FINAL REPORT

IDENTIFICATION AND MANAGEMENT OF MULTIPLE THREATS TO RARE AND ENDANGERED PLANT SPECIES

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14. ABSTRACT

We investigated effects of deer, nutrient addition, and presence of non-native plants, earthworms and a root-weevil on demography of rare understory species (*Aristolochia serpentaria, Agrimonia rostellata, Carex retroflexa*, and *Trillium erectum*) using a combination of experimental manipulations and mark-recapture observations at US Army Garrison West Point, NY. After developing stage-specific matrix projection models, we conducted life table response experiments to estimate how different stressors affect contributions of different vital rates to changes in population growth rates. Density and species composition of monitored stressor organisms varied across field sites and years. Marked individuals of all species had a positive response to fencing: overall individuals of all species grew taller, with larger leaves and had higher probabilities of flowering or seed production. Population growth rate (λ) of three of our species (*A. rostellata, C. retroflexa* and *T. erectum*) was >1, indicating that populations are projected to grow. Nevertheless, λ for all species was higher in fenced than open plots. *Aristolochia serpentaria* growth rate was <1 in the open plot

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List of Acronyms

GLM	Generalized Linear Model
GLMM	Generalized Mixed Linear Model
LM	Linear Model
LTRE	Life table response experiment
MLM	Mixed Linear Model
SAR	Species at risk: *Agrimonia rostellata* Wallr., *Aristolochia serpentaria* L., *Carex retroflexa* Muhl. ex Wild., *Trillium erectum* L.

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Abstract

Objective: Forest ecosystems in eastern North America face multiple threats including habitat loss and fragmentation, invasions, overabundance of native species, nutrient deposition and climate change. While each stressor may have independent detrimental effects on native biota, stressors often co-occur and are likely to have synergistic effects. Our goal was to assess interactive effects of deer, nutrient addition, and presence of non-native plants, earthworms and a root-weevil *Barypeithes pellucidus* Boheman) on demography of rare understory plant species. We selected four plants species *Aristolochia serpentaria* L*, Agrimonia rostellata* Wallr*, Carex retroflexa* Muhl. ex Willd, and *Trillium erectum* L. (hereafter referred to as SAR), which differ in their predicted response to deer and earthworm activity.

Technical Approach: We used a combination of experimental manipulations and mark-recapture observations in the field to assess effects of deer, earthworms, slugs, root weevil abundance, and invasive plants to vital rates of SAR. After developing We developed stage-specific matrix projection models for each SAR and then conducted life table response experiments (LTRE's) to estimate how different stressors affect contributions of different vital rates to changes in population growth rates.

We conducted investigations at US Army Garrison West Point, NY (hereafter referred to as West Point) and in common gardens at Cornell University. We selected 12 field sites at West Point, which varied according to abundance of three target non-native plant species (*Alliaria petiolata* (M. Bieb.) Cavara & Grande*, Berberis thunbergii* DC and *Microstegium vimineum* (Trin.) A. Camus) and according to earthworm density (8 and 4 sites with high and low earthworm density, respectively). At each site we established two 30 x 30 m plots, one open and one protected by a deer-proof fence. From 2009-2012, we monitored vegetation, earthworm, slug and *B. pellucidus* populations at each plot. In order to estimate SAR recruitment and juvenile survival, we conducted a seed bank study, and seeding and transplant experiments. Over five years, we collected mark-recapture data of adult individuals in open and deer-fenced plots established in one extant population of each SAR. At Cornell University, we conducted slug feeding trials and common garden experiments to assess interactive effects of earthworm and *B. pellucidus* on SAR germination and survival.

Results: Density and species composition of monitored stressor organisms (earthworms, slugs and *B. pellucidus*) varied across field sites and years. Unexpectedly, we found that earthworm density and biomass decreased in the fenced plots, indicating a possible, but unforeseen, interaction between earthworms and deer.

In just five years, we found that all three target non-native plants had significantly lower abundance (frequency, cover and/or density) in fenced plots, in response to deer exclusion. This is particularly true for the short-lived *M. vimineum* and *A. petiolata,* which are annual and biennial, respectively. Simultaneously, native vegetation responded positively to deer exclusion. Results indicate that it may be possible to reduce abundance of non-native plants simply by substantially reducing deer density.

Contrary to our expectations, stressor impacts were not always negative and were even beneficial at certain life stages. For example, a common garden study indicated that earthworm presence, most likely through a reduction in leaf litter accumulation, increased germination of the majority of tested plant species. Similarly, *A. serpentaria* and *T. erectum* germination in field trials was significantly higher at sites dominated by the invasive *M. vimineum* than at native-

vegetation sites. Not surprisingly, we found that SAR response to stressors was species- and stage-specific. For example, while *M. vimineum* had a positive effect on germination of two SAR, it had no effect on survival and growth of transplanted seedlings of the same two SAR. Additionally, while fencing had a small effect on survival or growth of transplanted seedlings of all SAR, earthworm density had a distinct effect on each SAR: *A. rostellata* and *C. retroflexa* survival and growth were higher at high earthworm density sites, while *T. erectum* transplants did better at sites with low earthworm density and *A. serpentaria* seedlings were not affected by earthworm density.

Marked individuals of all species had a positive response to fencing: overall individuals of all species grew taller, with larger leaves and had higher probabilities of flowering or seed production. *Carex retroflexa* showed a tendency for higher growth (new culm production) in the open plot but for higher reproduction (seed production) in the fenced plot. This was the only SAR for which we did not detect any above-ground mammal herbivory in the open plot. For the three remaining SAR, browsing was significantly lower in the fenced plot. Attack of adult *A. serpentaria, A. rostellata* and *T. erectum* in the fenced plot was most likely due to rodent feeding as evidenced by the angular cut of stems. These three species also showed frequent attack by a variety of invertebrate herbivores.

Population growth rate (λ) of three of our species (*A. rostellata, C. retroflexa* and *T. erectum*) was >1, indicating that populations are projected to grow. Nevertheless, λ for all species was higher in fenced than open plots. *Aristolochia serpentaria* growth rate was <1 in the open plot and increased to just one after five years of fencing, indicating that deer effects might be of special relevance for maintenance of this threatened species. LTRE analysis indicated a greater effect of fencing compared to that of earthworm density, but effects manifested themselves through different life cycle pathways for each SAR.

Benefits: Biodiversity conservation projects should critically consider effects of multiple stressors; however, our results emphasize the importance of deer as a structural force and indicate that reductions in deer populations are imperative (and may be sufficient) to restore and preserve populations of rare plant species.

The ecological carrying capacity for local deer herds in different habitats is a function of vegetation composition, soil fertility, climate, previous land use history, and many other factors, including biological invasions. It is difficult for local land managers to separate effects of these different factors without detailed experimental manipulations. To address this problem we have developed a method using indicator species to assess deer browse pressure. In our study, "sentinel" oak seedlings (*Quercus rubra* L) protected from deer herbivory continued to grow while those exposed to deer suffered major mortality. This approach allows land managers who have identified conservation goals as the main objective of management activities to set deer population targets based on actual deer browse pressure, rather than relying on questionable historic deer abundance estimates, or hunter satisfaction surveys. This approach allows to assess local browse pressure (10 - 50 ha) but is not sufficient over larger landscapes. Furthermore, while we anticipate that in most areas of North America deer populations are too high to allow any oak recruitment, the protection of browse sensitive species will likely require large deer reduction efforts. It will require future development of different and more sensitive indicator species to assess deer browse pressure at lower deer densities. Until such reductions are in place vulnerable plant populations may need to be fenced to allow their recovery.

1. Introduction

1.1. Background

Plant species composition of eastern forests has undergone dramatic changes over the past 100 years, mostly driven by changes in human land use, particularly clearing large areas for agricultural purposes and selective cutting of valuable tree species. Many plant species now exist in isolated or small populations and are considered threatened, endangered or of special concern (SAR). Although abandonment of many farms in the past 100 years has resulted in a considerable increase in forest cover in the Northeast, re-colonization of forests by many understory species is slow or non-existent (Vellend, 2003). While past land use history is often considered the most important factor preventing native species from re-colonizing these "new" forests (Vellend, 2003), the potential for biotic changes in plant and animal communities to drive these observed patterns has been rarely considered.

Eastern North American forests have seen dramatic increases in white tailed deer (*Odocoileus virginianus* Zimmermann) populations and invasions by nonnative plants, invertebrates and diseases. Notorious and visible diseases such as chestnut blight and beech bark disease, and invertebrates such as gypsy moth and hemlock wholly adelgid cause large-scale tree mortality and changes in forest tree composition. Eastern forests have also been invaded by numerous other species that are less visible such as earthworms (Bohlen et. al., 2004b), slugs (Chichester and Getz, 1969) and the root feeding weevil *Barypeithes pellucidus* (Boheman) (Maerz et. al., 2005c). Invasive plants such as *Berberis thunbergii* DC, *Alliaria petiolata* (M. Bieb.) Cavara & Grande and *Microstegium vimineum (Trin.) A. Camus*), are widespread in eastern forests and often considered a major threat to SAR. These changes in biota plus general habitat loss and fragmentation, acid rain, nutrient deposition and climate change may all threaten the long-term survival of SAR. Abundant evidence for the "ecosystem engineering" ability of nutrient deposition, deer and slug herbivory and plant and earthworm invasion exists; but there has been no attempt to combine these different stressors into a single study or model. This is what we attempted to accomplish with the work at US Army Garrison West Point installation.

Ideally, any management of plant populations or of various other threats would be informed by an analysis of the severity of a particular threat or of the cumulative threat posed by various stressors and how a particular plant species/population responds to management. Unfortunately, such guidelines are not available to land managers at military installations or elsewhere. Assessments of the relative contribution of these various stressors to several SAR plant species and their demography will not only illuminate the severity of single or combined threats but also allow formulating management recommendations. In the following we will first outline the existing knowledge of individual stressors and the theoretical and conceptual framework that has guided our work over the past five seasons before reporting on our results.

1.1.1. Deer, slugs, earthworms, invasive insects and plants and nutrient deposition as ecosystem engineers

1.1.1.1. Deer herbivory

Historically, white-tailed deer populations in eastern North America were estimated at 3-5 deer/km^2 but numbers have increased an order of magnitude over the past 100 years (Fletcher

et. al., 2001). Habitat changes, elimination of predators and changes in hunting regulations, have all contributed to this remarkable population explosion. Foresters and botanists have for decades documented detrimental impacts of increased deer browse (often through exclosure studies) on forest tree regeneration and understory herbaceous vegetation (Alverson et. al., 1988; Miller et. al., 1992; Anderson and Katz, 1993; Porter and Underwood, 1999; Horsley et. al., 2003; Rooney and Waller, 2003; Ruhren and Handel, 2003; Kraft et. al., 2004; Webster et. al., 2005). Intense selective deer herbivory creates a shift in plant species composition towards browse resistant or unpalatable species (Horsley et. al., 2003). Detailed studies consistently show that species in certain functional groups such as grasses, sedges and some ferns are increasing under intense deer herbivory, while overall herb diversity is declining (Horsley et. al., 2003; Rooney and Waller, 2003). Orchids, *Trillium* spp. and other liliaceous plants appear particularly sensitive to deer browse (Miller et. al., 1992; Gregg, 2004; Webster et. al., 2005). In addition to direct effects on individual plant species and successional dynamics, deer herbivory creates cascading effects in forest ecosystems and food webs affecting birds (McShea and Rappole, 1999), nutrient cycling, and decomposer food webs (Rooney and Waller, 2003; Wardle and Bardgett, 2004).

Detailed demographic studies of *Trillium grandiflorum* (Michx.) Salisb. (Knight, 2004) and ginseng, *Panax quinquefolius* L. (McGraw and Furedi, 2005) show deer herbivory to be the single largest factor affecting population growth rates (these studies only considered harvesting (*P. quinquefolius*) and pollen limitation (*T. grandiflorum*) as alternative factors). Demographic analyses revealed that a reduction in deer herbivory shifted both herbs from negative to positive growth rates. Browse rate was consistently most pronounced for adult plants (11-100%) (McGraw and Furedi, 2005). This feeding pattern effectively removed the largest individuals, which contribute most to population growth rates from the population (Augustine and Frelich, 1997; Knight, 2004; McGraw and Furedi, 2005). This scenario is particularly troubling for species that currently appear to be safeguarded and do not have current SAR or SAR status. For example for ginseng population viability is a function of initial population size, and models shows that most censused populations are already too small to prevent extinction at the current levels of browse (McGraw and Furedi, 2005). An important goal of management of fauna and flora at military installations should be recognition of species that may be candidates for future listings and implement preventive measures to prevent population declines. Our approach will therefore incorporate 1-2 plants not currently listed but favored by deer into our demographic modeling.

1.1.1.2. Earthworm invasions

Invasions of European and Asian earthworms into historically earthworm free temperate North American forests have been long overlooked and only recently have data on their impacts emerged (Hendrix and Bohlen, 2002; Bohlen et. al., 2004a; Bohlen et. al., 2004b; Hale et. al., 2005a). Multiple species with different life-histories have been introduced and epigeic species (residing in the upper organic layer causing limited mixing of mineral and organic layers), endogeic species (residing in the mineral or mixed soil layers often enhancing mixing of these layers) and anecic species (residing in vertical burrows 1-2m deep, incorporating litter into the soil and creating a deep soil mixing) appear to affect forest floor communities in different ways (Bohlen et. al., 2004b). In general, earthworms shift the soil system from a slower cycling, fungal-dominated system to a faster cycling, more bacterial dominated system (Bohlen et. al., 2004b). Earthworm activity changes carbon, phosphorous, and nitrogen cycling, and affects the physical and geochemical structure of the forest floor (reduced leaf litter layer, increased mixing, deposition of castings, increased erosion). We expect that changes in nutrient dynamics may

affect the nutrient content of plants and therefore palatability and attractiveness to herbivores. These effects are most likely species-specific as has been demonstrated in Minnesota where foliar nitrogen content for *Carex pensylvanica* Lam., *Acer saccharum* Marshall and *Asarum canadense* L. decreased while foliar nitrogen for *Athyrium filix-femina* L. (Roth) increased (Hale et. al., 2005b).

The ecological effects of earthworm invasions include changes in resource quantity and quality for plants, loss of native plant species, increased invasion of nonnative plant species, and shifts in soil invertebrate and microbial communities (Bohlen et. al., 2004b; Hale et. al., 2005a). Many native plant species disappear as earthworm invasions progress (Gundale, 2002; Bohlen et. al., 2004b). Earthworms may cause direct mortality of plants by exposing roots in the rapidly disappearing organic layer. Seeds may suffer increased mortality due to desiccation or predation because earthworms remove the protective leaf litter layer or directly eat or bury propagules. Changes in nutrient dynamics may also affect germination and seedling establishment. In addition, earthworms consume mycorrhizae (Lawrence et. al., 2003) and this may explain why non-mycorrhizal species such as *C. pensylvanica* and *Arisaema triphyllum* L. (Schott) appear able to maintain populations in the presence of invasive earthworms (Bohlen et. al., 2004b). In addition, many European and Asian plant invaders are non-mycorrhizal and thus appear well adapted to coexist with introduced earthworms.

Our own previous work has shown (Maerz et. al., 2009; Nuzzo et. al., 2009) that the importance of nonnative plant invasions (*A. petiolata*, *B. thunbergii*, and *M. vimineum*) on forest plant communities, and invertebrate and salamander populations in the Northeast is small in comparison to the effect of introduced earthworms. We worked across invasion fronts to capture potential changes associated with advancing invasive plant populations. We could not detect a clear signal for impact of nonnative plants on native plant species richness or abundance, nor on salamander populations. Instead, we found that nonnative earthworm biomass was significantly greater in invasive plant habitats (Nuzzo et. al., 2009). Furthermore, the increased abundance of invasive earthworms was positively correlated with accelerated loss of the organic layer where many shrubs and perennial species root. While the impact of worm invasion is reported to vary depending upon worm species and characteristics of the invaded habitat (Bohlen et. al., 2004b; Hale et. al., 2005a), we found a clear trend for all invaded sites to respond similarly to worm invasion, despite differences in worm species and abundance, and distinct differences in composition of the invaded communities, and the identity of the invasive plant species. The loss of the organic leaf litter layer resulted in drastic changes in forest floor invertebrates and changes in prey abundance, which negatively influenced salamander abundance. We concluded that worm invasion, rather than nonnative plant invasion, is the driving force behind changes in forest floor community composition, although there appeared to be an interaction of invasive plants and invasive worms. However, we also realize that native plant communities can be diverse and reach high cover in the presence of invasive earthworms, contradicting results by others (Hendrix and Bohlen, 2002; Hale et. al., 2005a; Hale et. al., 2005b). We hypothesize that these differences may be the result of longer invasion histories in the Northeast, where native plant communities have had time to recover after initial earthworm invasions. We cannot entirely exclude the possibility, however, that the collapse of plant populations as a result of invasive plant and earthworm invasions is only a matter of time and may take longer in the Northeast than in Minnesota. Importantly, our experiments on the impacts of invasive plants on forest communities, and many of the studies we cite, did not manipulate deer access. With deer being recognized as an important contributor to change in forest communities we cannot exclude the possibility that many of the patterns described and recognized in Eastern forests are at least in part or to a large extend shaped by overabundant white tailed deer.

1.1.1.3. Slug herbivory

Slugs are important generalist herbivores affecting ground layer vegetation and can influence both the biomass and species composition of plant communities (Buschmann et. al., 2005b). Despite being considered polyphagous, slugs show distinct feeding preferences, which result in shifts in dominance among plant species (Buckland and Grime, 2000; Buschmann et. al., 2005b; Peters et. al., 2006). Whereas voracious and abundant slugs are prevalent in Europe, high levels of slug herbivory in the eastern and central US are a more recent phenomenon and mainly due to *Arion* spp. and *Deroceras reticulatum* (Müller) (Buschmann et. al., 2005a). Species in both genera were introduced approximately 150 years ago and are now spreading rapidly (Chichester and Getz, 1969; Pinceel et. al., 2005). Studies in boreal forests indicate that non-native species might comprise more than 90% of slug captures, with *A. subfuscus* (Draparnaud) being the most frequent species (Ferguson, 2004; Moss and Hermanutz, 2010). *Deroceras reticulatum*, for example, was prevalent and abundant at all our field sites surveyed for the impact of invasive plant species on salamanders (Maerz et. al., 2009). Slug herbivory primarily affects seedling survival, while established plants are more likely to survive slug grazing, although their vigor and fitness may be reduced (Ehrlen, 1995). In European grasslands, slug grazing may increase plant diversity by reducing the vigor of dominants, thereby allowing less competitive species to colonize (Buschmann et. al., 2005b), but the effect of non-native slug herbivory on plant community composition in North American forests is entirely unknown. In New Zealand introduced *D. reticulatum* had strong negative impacts on a native plant species and its recruitment (Sessions and Kelly, 2002). The invasion of an introduced European grass greatly accelerated slug impact in New Zealand (Sessions and Kelly, 2002) and similar invasional meltdowns (Simberloff and Von Holle, 1999) may occur in North America.

1.1.1.4. Non-native plant invasions

Invasion of non-native plants is considered a major threat to native biodiversity; non-native plants can displace native species, alter ecosystem function, and change trophic structure of invaded ecosystems (Wilcove et. al., 1998; Mack et. al., 2000). Our own work suggests that invasive species such as *Lythrum salicaria* L. or *Fallopia* x *bohemica* negatively affect American toads (Brown et. al., 2006) and green frogs (Maerz et. al., 2005a). Differences in chemical composition and decomposition rates of native and introduced wetland plants also alters the structure of amphibian communities (Maerz et. al., 2005b). However, while the potential impact of invasive plants in general has received much attention from managers and ecologists, there is little quantitative long-term evidence for the negative ecological impacts for most invasive plants (Blossey, 1999). Lack of evidence is no proof for lack of ecosystem impacts and may be associated with difficulties monitoring for such effects over extended time periods (Blossey, 1999). For example, successful biological control of *L. salicaria* resulted in the return of many native plant and animal species where they were previously absent or rare (Blossey et. al., 2001). This recovery of native species suggests a strong negative impact of the invader on native plant and animal communities. However, some recent evidence suggests that invasive plant species may be passengers and not the drivers of ecosystem change (MacDougall and Turkington, 2005). In our own work we failed to detect impacts of *A. petiolata* on ground beetle composition and foraging (Dávalos and Blossey, 2004). Invasive forest plant species appeared to have little effects on ground layer vegetation (Nuzzo et. al., 2009) and salamander populations in eastern forests (Maerz et. al., 2009). Rather, earthworms were the driving force and in our surveys introduced plant species were always associated with earthworm-infested soils, while earthworms commonly occurred in the absence of invasive plants.

1.1.1.5. Nutrient deposition

Large-scale industrialization and intense agriculture have increased deposition rates of essential nutrients for plant growth across the globe. Increased nutrient availability, particularly of nitrogen and phosphorous, results in changes in the response of individual plants (some increase, others decrease in their competitive ability) and these responses may vary depending on plant-specific traits (Keddy et. al., 2000; Gross et. al., 2005). A recent global analysis suggests that rare plants in particular appear favored by low phosphorous levels (Wassen et. al., 2005). These changes in nutrient deposition rates affect plant community dynamics (Britton et. al., 2001), and litter nutrient composition with extended impacts on detrital food webs (Miki and Kondoh, 2002). And perhaps most importantly, changes in nutrient status of the soil, either through deposition or through earthworm invasions, affect plant nutrient composition. These changes affect plant defense chemistry, change herbivore resistance (Glynn et. al., 2003), affect fruit removal (Schaefer et. al., 2003) and change the palatability of plants to slugs (Albrectsen et. al., 2004).

1.1.1.6. *Barypeithes pellucidus* herbivory

One of the nearly completely overlooked insect invaders in northeastern forests is the root weevil *B. pellucidus*. Larvae of the weevil live belowground where they feed on tree and herbaceous plant roots. Root feeders can have important consequences for individual plant performance and plant population dynamics and many species have been successfully used as weed biocontrol agents (Blossey and Hunt-Joshi, 2003), but we have absolutely no information on the effects of *B. pellucidus* on plant growth or fitness. Our investigations revealed that adult spring densities can exceed of 100 individuals/m^2 and *B pellucidus* has become an important prey item for salamanders (Maerz et. al., 2005c). Such densities suggest that the species may have important impacts on its host plants and this attack may also affect aboveground herbivores (Blossey and Hunt-Joshi, 2003).

In summary, the individual effects of increases in deer and slug herbivory, changes in nutrient deposition, as well as insect, earthworm and nonnative plant invasions on plant community composition have received considerable attention. Sorely lacking is a threat assessment of the contribution of these different stressors (singly and in combination) to decline of native plant species and their listing as SAR and the integration of this information into comprehensive management plans. Ideally, any management of plant populations or of various other threats would be informed by an analysis of the severity of a particular threat or of the cumulative threat posed by various stressors and how a particular plant species/population responds to management. Unfortunately, such guidelines are not available to land managers at military installations or elsewhere. Assessments of the relative contribution of these various stressors to several SAR plant species and their demography will not only illuminate the severity of single or combined threats but also allow formulating management recommendations.

1.1.2. Ecosystem impacts of deer, slugs, earthworms and invasive plants: a hypothetical scenario

In our studies we were guided by the following hypothetical scenario that we anticipate is being played out across much of Eastern and Midwestern North America. Native plant species, through habitat fragmentation or specialized habitat requirements, have undergone substantial declines. The most vulnerable to multiple stressors or changes in habitat conditions are now

listed as SAR species. Increasing deer abundance over the past 100 years suppresses growth and reproduction, and reduces survival of long-lived species. Earthworm invasions change the physical and biogeochemical properties of the forest floor, often resulting in large-scale demise (at least initially) of the forest understory plant communities. After the initial earthworm invasion front has passed, many native plant species are able to recover (as evidenced in deer exclosures), however, seedling establishment is difficult due to lack of a seed bank (Lambers et. al., 2005), and increased herbivory by introduced slugs or insects. If seeds successfully germinate and establish, deer herbivory prevents the majority from ever becoming reproductive adults. Invasive plants colonize the empty space created by deer herbivory and earthworm invasions, and further limit native plant species through direct resource competition.

In our scenario, deer herbivory is the major structuring force overwhelming (nearly) every other threat to SAR. Deer herbivory not only threatens survival and existence of many native plant species, but also facilitates plant invasions by reducing competition by and biotic resistance of native plant species. Earthworm invasion is the second largest threat and is initially devastating to native plant populations that may also lose their competitive advantage through changes in nutrient levels. In the presence of high deer populations, slugs (through direct consumption) and introduced plant species (through competition) prevent recovery of the forest floor plant communities from the seed bank. We have evidence for this scenario through our work in eastern forests (Nuzzo et. al., 2009) and long-term vegetation monitoring (Nuzzo, 2004) at Fermi Laboratory in Batavia IL, where >90% culling of the deer herd occurred in 1998. This cull and an attempt to stabilize the deer herd at about 4/km^2 increased ground cover of native plant species from 15% to 62%, decreased cover of invasive plant species from 32% to 19%, increased average vegetation height from 13cm to 42cm and allowed native plant species to return or regain reproductive status (from 7% to 22%), in the presence of invasive plants (garlic mustard) and earthworms (Nuzzo, 2004). This substantially reduced deer density still has an impact on highly preferred species; for example *Maianthemum racemosum* L. (Link) is heavily browsed and populations have not recovered 7 years after the deer cull.

1.2. Objective

Our work developed in response to a Statement of Need (SISON-07-01) asking for the development of scientific methods for assessing cumulative effects of multiple stressors on threatened and endangered species (TES) and species at risk (SAR). The desired outcome was improved quantitative ecological forecasting abilities through models that inform decision makers on managing stressors affecting TES. Ideally, any management action should be backed up by quantitative information about effect sizes of single and combined stressors, but such information is rarely available to land managers at military installations and elsewhere. This leads to problematic decision making processes and ultimately questionable or poor management outcomes. Our particular work focused on multiple stressors in Eastern US forests (deer, invasive plants, slugs, earthworms and a root weevil), as well as influences of nutrient deposition on plant demography of four woodland species. Identification of additive, synergistic or even lack of effect of individual stressors or stressor combinations should help focus on appropriate management regimes that would safeguard species at risk, ultimately saving resources otherwise spent on potentially wasteful management actions and retaining the ability for military training.

We selected species in different life forms and functional groups, as well as with known or expected differences in vulnerabilities to our selected stressors. In responding to the SON,

our main interest was in exploring the relationship between changes in one or more vital rates and the population dynamics of various TES, in response to various agents (e.g. invasive plants, worms, slugs, invasive insects, deer browsing or increased nutrient deposition). While it would seem intuitive that the most appropriate metric to characterize population dynamics for a given TES is abundance (population size), estimation of abundance in open populations is notoriously difficult, even for sessile taxa. Moreover, abundance is itself the product of some starting population vector, and successive changes (growth or decline). These changes are characterized by annual growth rates. Recently, however, there has been recognition that identifying contributions to population change may be more usefully assessed in terms of contribution of the vital rates to variation in *projected* growth rate, rather than abundance (Caswell, 2000). In particular, projected growth rate is an omnibus *index* of the cumulative contributions of the underlying birth and death processes. Moreover, methods relying on analysis of projected growth rate are not affected by difficulties due to issues of age or size structure and post-reproductive individuals (which is a common problem with vertebrate species). We apply approaches to partitioning variation in population trajectory to variation among one or more underlying vital rates.

At the beginning of our project we were guided by the following working hypotheses:

- Effects of overabundant white-tailed deer override effects of any other stressor
- The overwhelming effect of deer is due to their selective foraging on preferred species, especially the largest, most reproductively active individuals. Deer exclusion results in rapid plant community recovery (species abundance and diversity).
- Invasive plants (*Alliaria petiolata*, *Berberis thunbergii* and *Microstegium vimineum*) play a minor role in the endangerment of plant species.
- Invasive earthworms benefit graminoids, and may contribute to endangerment of forbs.
- Introduced slugs, root weevils and nutrient addition may affect seedling establishment but their effects contribute little to endangerment of established plants.
- Restoration efforts will be successful if seeds and seedlings are protected from deer herbivory.

2. Materials and Methods

2.1. Field site selection

We worked at US Army Garrison West Point (hereafter referred to as 'West Point"), a 65km^2 facility located on the west bank of the Hudson River, approximately 80 km north of New York City, NY, USA (**Fig. 1**). West Point is located within the Hudson Highlands Province, characterized by rugged hilly terrain with rocky outcrops and frequently thin soils. We selected 12 upland deciduous forests > 1km apart, avoiding areas with high training intensity and/or restricted access. Forests are dominated by oak (*Quercus rubra* L. and *Q. prinus* L.) and/or sugar maple (*Acer saccharum*) but differ in land use history, aspect, soil, and plant species composition. We selected 6 sites based on the presence and abundance of three non-native plant species (*Alliaria petiolata, Berberis thunbergii, Microstegium vimineum*; 2 sites each) and 6 sites with few or no non-native plant species present (**Table 1**). At each site we established two 30m x 30m plots (one fenced and one open; one of each pair of plots was randomly assigned to receive fencing or, in some cases, was selected for fencing based on presence of corner trees at the correct distance; **Fig. 2**). Paired plots were 5-50m apart. We erected deer-proof exclosures (millennium plastic deer fence, 2.6m high fence, deerBusters.com) from 7 to 11 July 2008.

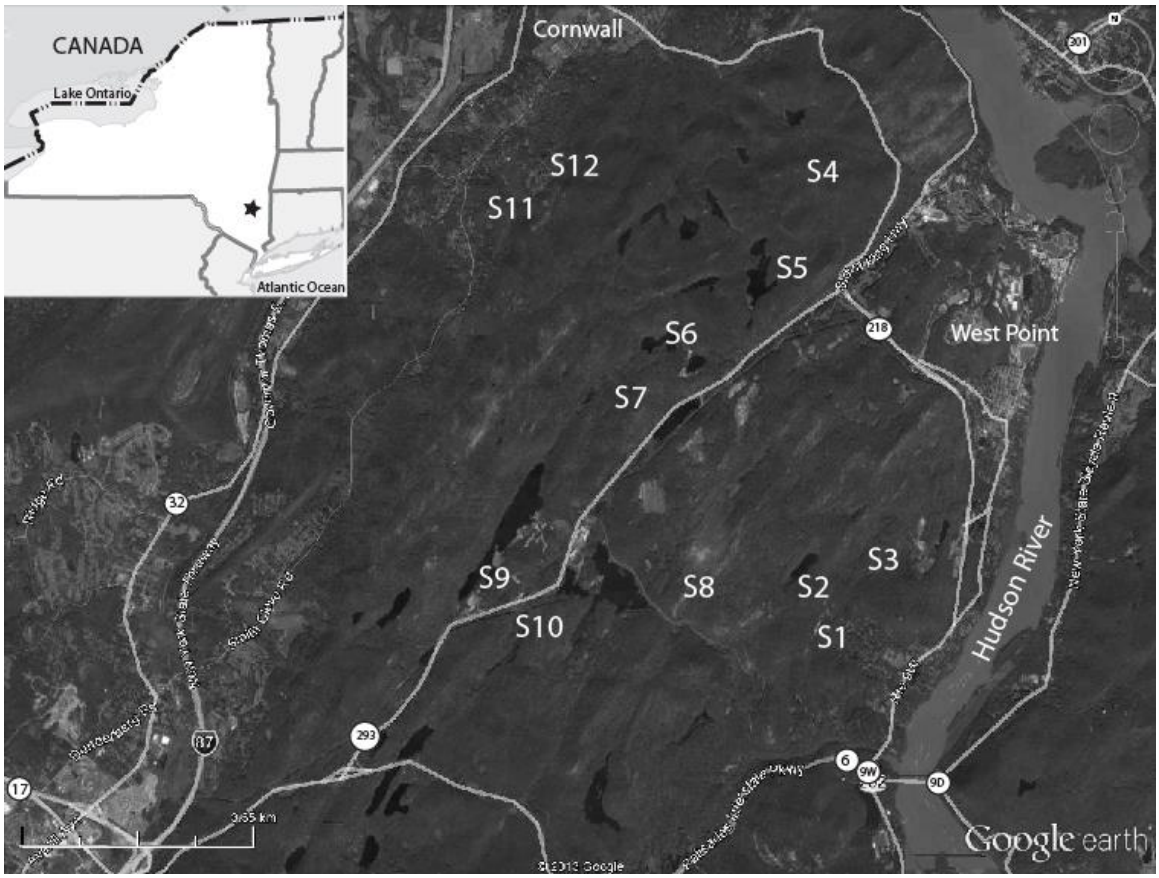

Fig. 1. Map showing the location of the study area in New York State (star indicates location on inset map) and a Google Earth image of the 12 study sites at West Point, NY. For site characteristics see Table 1.

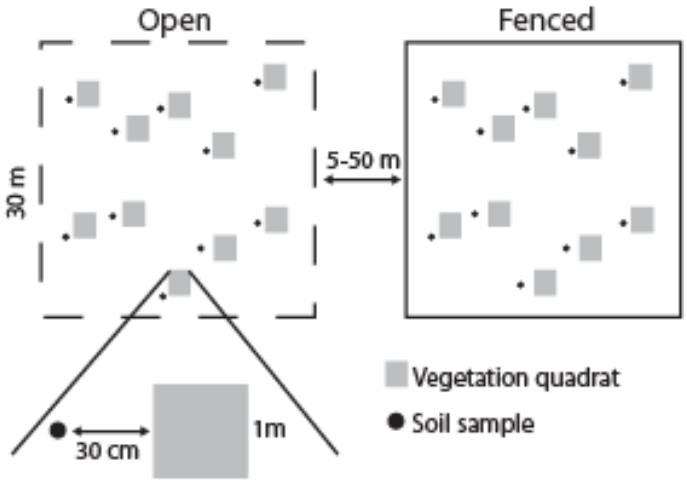

Fig. 2. Experimental design showing paired open and deer-fenced plots and approximate location of permanent vegetation quadrats (grey squares) and soil core samples (filled circles). The study was conducted at 12 sites at West Point, NY. Graph is not drawn to scale.

Table 1. Locations and main characteristics of 12 sites (each with an open and fenced plot) at West Point, 2008. Six sites are dominated by non-native plants (*A. petiolata, B. thunbergii* and *M. vimineum*, 2 sites/species) and six lack invasive plants (None (a) and (b), 3 sites each). Several sites (a) were expected to have few worms present when selected based on observations, while others (b) were known to have worms present.

Site	Non-native Plant	Non-native Plant cover (% in 2008)	Earthworm density
1	*Berberis thunbergii*	21.3%	Low
2	None (a)	--	Low
3	*Microstegium vimineum*	37.1%	High
4	None (a)	--	Low
5	*Alliaria petiolata*	4.2%	High
6	*Berberis thunbergii*	95%	High
7	*Alliaria petiolata*	5.7%	High
8	None (a)	--	High
9	None (b)	--	High
10	None (b)	--	High
11	None (b)	--	Low
12	*Microstegium vimineum*	96%	High

2.2. Soil analyses

At each site we collected one soil core approximately 30 cm from each permanent quadrat (see section 2.4.1 for details; **Fig. 2**), first removing loose leaf litter and then using a 7 cm diameter one piece auger (Ben Meadows) to a depth of 5 cm (12 sites X 2 fence treatments X 10 replicates = 240 soil cores). Cores were collected 28-31 October 2008, stored in plastic bags and kept in a cooler until sent for analysis. We pooled the 10 soil samples collected from within

each fenced or open plot, mixed the soil well, removed two separate subsamples (0.25 l)/plot, and submitted samples for soil analysis to the Cornell Nutrient Analysis Laboratory in early 2009 for pH, exchangeable acidity, organic matter (LOI), Morgan extractable P, K, Ca, Mg, MN, Zn, Al, NO_3, total carbon and total nitrogen.

2.3. Seed bank study

We used a well-mixed portion of soil collected for soil analyses (see section 2.2) and removed rocks, large roots, and leaves from each soil sample for the seed bank study. Collecting soil cores in October 2008, after seed set of all species was complete for the current year, allowed us to record both the transient and persistent seed bank. Initial seed bank composition should not be affected by the fence, as we detected no initial difference in flowering or fruiting frequency in fenced and open plots. On 1 November 2008 we placed the soil ~ 1 cm deep over a 3 cm layer of moistened sterile potting mix (Farfard Canadian growing mix No. 1-P, Agawam, Massachusetts, USA) in a plastic tray (12 x 8 x 5 cm), tamped the soil to remove air pockets, and watered the tray. We then placed trays into 20 flats (one sample/site/flat). We prepared 10 trays containing only potting mix as blank controls and distributed them among the seed bank flats to assess potential contamination from the planting mix; two species were recorded in all 'blank' trays, and these germinants were excluded from counts in the 120 seed bank trays.

All flats were kept outdoors in screened cages under a forest canopy to emulate 'natural' forest conditions (temperature, precipitation and light) and to prevent further seed addition (through wind) or removal (for example by rodent granivory or invertebrates). We moved flats in cages into a 1m deep north-facing coldframe each November/December after the seed bank samples were frozen, and removed them in April once nighttime temperatures remained above freezing. We added supplemental water only if trays were obviously dry and no rain had fallen for >2 weeks. We did not stir the seed trays at any time during the study to ensure that slow-germinating seeds, including those with epicotyle and radicle dormancy, would have the opportunity to emerge. Thus, we modified the seedling emergence method (Brown, 1992) by: 1) including seasonal temperature variation and thereby allow emergence of species with germination requirements not met by greenhouse conditions; 2) omitting repeated soil disturbance; and 3) monitoring germination beyond one year, to more fully capture the viable soil seed bank.

We recorded seedling emergence at monthly intervals between April and October 2009 and at bimonthly intervals in 2010 and 2011. Seedlings were removed as soon as they reached an identifiable stage; for different species this ranged from the cotyledon stage to flowering. Unidentified germinants were left in the seed trays to mature.

We used structural equation modeling (SEM) to examine the direct and indirect importance of three mechanisms: (1) plant invasion (non-native plant cover), (2) vegetation diversity (species richness and percent cover) and (3) earthworm abundance on the abundance and species richness of seed bank germinants. The SEM analysis was based on a conceptual model built under the following premises: (1) earthworm density and non-native plant cover are positively correlated (Nuzzo et. al., 2009), and both may directly influence germinant abundance and species richness (Forey et. al., 2011); (2) vegetation diversity will positively affect abundance and richness of germinants; (3) responses will differ according to origin (native or non-native) and life form of germinants, specially depending on whether the germinant is a graminoid or not. Using the function sem in the R (R Core Team, 2012) package lavaan (Rosseel, 2012), we fitted a theoretical model for each of the four identified groups: graminoid

and non-graminoid of native and non-native origin. We evaluated the adequacy of selected models by testing if there were no significant differences (P>0.05) between the likelihood of the model and data (via a chi-square test, Grace, 2006; Grace, 2008). We evaluated differences in model parameters across groups by sequentially constraining them to be equal (Grace, 2006; Rosseel, 2012) and used the Akaike Criterion (AIC) to evaluate the explanatory power of competing models (Burnham and Anderson, 2002) (see statistical analyses section 2.10).

We compared the seed bank community among levels of site invasion and earthworm density (low and high) with Permutational Multivariate Analysis of Variance (PerMANOVA). PerMANOVA is a non-parametric multivariate analysis of variance test that employs a permutation procedure. We performed 1000 permutations and used the Bray-Curtis method to calculate distance matrices. To fit the PerMANOVA we used the function adonis in the vegan package (Oksanen et. al., 2012; R Core Team, 2012).

We compared seed bank diversity and evenness among sites with Shannon (H'), Simpson (λ), Pielou (E_1) and Sheldon (E_2) indexes ($H' = \sum_{i=1}^{S}(p_i)ln(p_i)$; $\lambda = \sum_{i=1}^{S} p_i^2$; $E_1 = H'/\ln(S)$; $E_2 = e^{H'}/S$; where S= species richness, N= total abundance of germinants or vegetation cover, n_i= abundance or cover of the ith species and $p_i = n_i/N$). We used species density to estimate indexes for seed bank germinants and species cover for aboveground vegetation indexes. We estimated correspondence between species composition in the seed bank and aboveground vegetation using Jaccard's similarity index. We ran a two-way ANOVA to test if correspondence between seed bank and vegetation varied according to site invasion, life form, origin of germinants and earthworm density. Differences among life forms were evaluated with a posteriori Tukey HSD comparisons (alpha=0.05). We excluded unidentified species from diversity indexes calculations.

2.4. Field monitoring

2.4.1. Vegetation monitoring

We established 10 1-m^2 permanent quadrats in a stratified random manner in each fenced and open plot (**Fig. 2**) in July 2008 (11 sites) or May 2009 (1 site) (12 sites X 2 fence treatments X 10 replicates = 240 vegetation quadrats). Within each quadrat we recorded species presence, and estimated the percent cover for each species < 1 m tall in 17 cover categories (midpoints: 0.01, 0.2, 0.5, 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 98, and 100%). Additionally, we scored each species for presence/absence of mammal herbivory, and flower/fruit production. Plant species nomenclature for SAR and species found in the permanent quadrats follows the PLANTS Database (USDA NRCS, 2013). We recorded data twice a year, in mid-May and late July, from July 2008 – 2012. We estimated leaf litter volume by measuring the depth of the litter at four locations where litter was present within each quadrat, and then multiplying by the proportion of the quadrat covered by leaf litter. We estimated vegetation height by measuring the 'average' height of vegetation at four locations within each quadrat, and then averaging these heights for each quadrat.

We used cover class midpoint values for each species, and summed cover of all species within a quadrat; thus, total cover could exceed 100% due to layering of different plant species. We classified each species by status (native or non-native) and life form (annual and biennial forb, perennial forb, graminoid, Pteridiphyte, and woody). Graminoids included all grass-like plants (Poaceae, Cyperaceae and Juncacaeae).

Analyses were composed of three major parts: (1) we evaluated the effect of fencing and earthworm density on species richness, vegetation diversity, vegetation height, vegetation percent cover (native and non-native plant species), and leaf litter volume per quadrat with independent GLMMs for each response variable, including site and plot within site as random factors. We used Normal errors for all variables, except for species richness where we used Poisson errors. We evaluated vegetation diversity with Shannon (H'), Simpson (D) and Pielou (J) indexes (details in section 2.3). (2) We compared the herb layer community between the start (2008 for July samples and 2009 for May samples) and end (2012) of the study with Permutational Mutlivariate Analysis of Variance (PerMANOVA details in section 2.3). In this analysis we also evaluated the effect of fencing (open or fenced plot) and of earthworm density (low N=4, high N=8). We included plot identification as a stratum, to constrain permutations to each plot. (3) We examined species-specific shifts with log-likelihood ratio goodness of fit tests (*G*-test) to determine species that increased (winners) or decreased (losers) in frequency between the start and end of the study (Wiegmann and Waller, 2006). We only included species that appeared in at least 10% of quadrats for PerMANOVA and *G*-test analyses (25 species for May and 28 for July). We included all identified species and morpho-species to estimate species richness per plot, but we excluded morpho-species for the remaining analyses, as their classification was not consistent between sites. We used separate analyses for May and July surveys.

2.4.2. Plant vigor

We evaluated the effect of fencing on plant size and reproduction of three non-native plant species (*Alliaria petiolata, Berberis thunbergii* and *Microstegium vimineum*) and three native perennial plant species favored by deer (*Eurybia divaricata* (L.) G.L. Nesom, *Maianthemum racemosum* and *Polygonatum pubescens* (Willd.) Pursh). The study was conducted June-September 2012 at a subset of the 12 study sites established at West Point NY (see section 2.1). We assessed all sites for presence of the target species, but collected data only at sites where the target species were present (non-native plant species) or where we could find a minimum number of individuals in both open and fenced plots (25 for *M. racemosum* and *P. pubescens* and 50 for *E. divaricata*).

2.4.2.1. *Alliaria petiolata*

In mid-June 2012 we collected data on *A. petiolata* vigor in 20 randomly located 1m radius circular quadrats at three sites, recording density/quadrat, and measuring height and number of siliques of each individual. We then collected all ripening seeds from each individual to determine seed output/plant. Plants in two fenced plots (sites 5 and 7) occurred at low density, and to collect additional plants we walked 1-2 randomly established transects until we encountered an *A. petiolata* plant; we then established an additional 1m radius circular quadrat placed to encompass as many plants as possible, and measured each plant within the circular quadrat until we reached our target goal of 50 plants total (5 additional quadrats at Site 5, 4 at Site 7, and 3 at Site 12). We then added the same number of additional quadrats (similarly located along random transects) in the paired open plots to ensure equal sampling effort. Thus, we recorded density in the initial 20 1m radius quadrats, and mean height and seed output in both the initial and additional quadrats.

2.4.2.2. *Berberis thunbergii*

We compared annual woody growth of *B. thunbergii* in open and fenced plots at eight of the 12 study sites using growth rings. In June 2011, we randomly sampled 232 *B. thunbergii* shrubs: 10-16 per open and fenced plot depending on the number of available shrubs that fitted our criteria. The summary of samples taken at open and fenced plots and at sites with low and high earthworm density is detailed in **Table 2.** We selected shrubs at least 1 m tall and separated from other individuals by >1 m and then collected a basal stem disc from the widest stem. We sanded and polished a cross section of each sample in order to accurately measure growth rings (**Fig. 3**). We measured ring width along two radii from the pith and outward to the nearest 0.001 mm over a measuring platform set under a microscope. We conducted all measurements under the Tellervo© application (Brewer, 2011) with technical support provided by The Malcolm and Carolyn Wiener Laboratory for Aegean and Near Eastern Dendrochronology at Cornell University. We used the dplR library to conduct dendrochronological analyses (Bunn, 2008; Bunn et. al., 2012). We first transformed tree ring width data to basal area increment (BAI) because area represents tree growth better than a linear measurement (Biondi and Qeadan, 2008). We standardized the age trend (ring width decreased with age) via a ln regression of BAI on age: $\ln(BAI) = a + b \times \ln(Age)$ (**Fig. 4**). We evaluated the effect of fencing on the residuals from the previous model with a linear mixed model, which included shrub identity, within plot and site as random factors. We assumed that all measurements prior to 2008 correspond to the open "treatment".

Table 2. Summary of tree-ring series across study factors

Factor	N sites	N shrubs	Time span	Mean age (SD)
Fenced	8	114	1994-2011	7.97 (4.28)
Open	8	118	1995-2011	7.85 (3.70)
High earthworm density	6	171	1994-2011	7.42 (3.84)
Low earthworm density	2	61	1995-2011	9.22 (4.10)

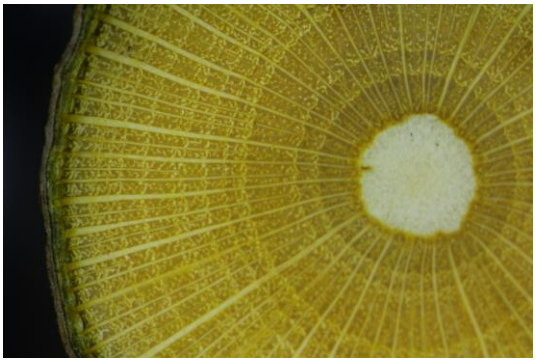

Fig. 3. Cross section of *B. thunbergii* stem showing annual growth rings.

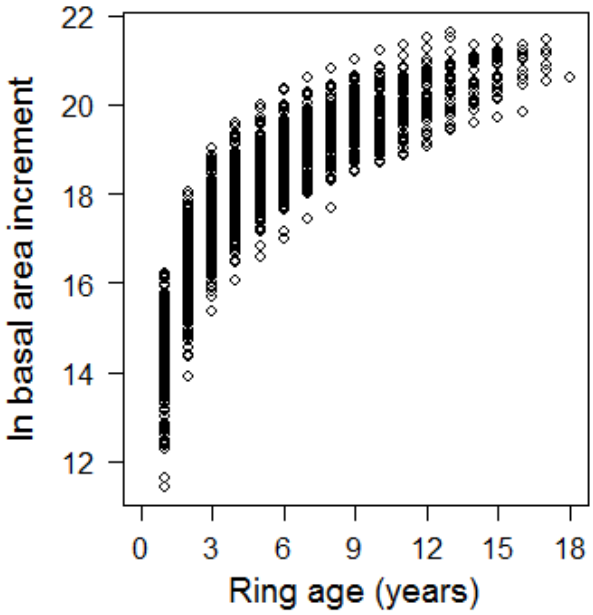

Fig. 4. Basal area increment (BAI) according to age (years) of *B. thunbergii* collected from open and fenced plots at 8 sites at West Point, NY. BAI were age-standardized using parameters estimated from a linear regression: ln(BAI) = 14.63 + 2.30 * ln(age), R^2=0.88, P<0.001. Data for open and fenced plots are aggregated.

2.4.2.3. *Microstegium vimineum*

In late August 2012 we collected data on Japanese stiltgrass (*M. vimineum*) vigor at three sites. We established ten 0.25 m^2 quadrats/plot, each quadrat adjacent to one designated corner of our vegetation quadrats. If *M. vimineum* was not present in the initial quadrat location, we searched for the closest *M. vimineum*, and then placed the quadrat with the plant in one corner and shifted to maximize capture of *M. vimineum* in the re-established quadrat. Thus, we recorded frequency in the initial quadrats, and plant vigor in both the initial and additional quadrats. We then collected all *M. vimineum* plants (shoots and roots) from each quadrat, kept plants separately for each quadrat and subsequently dried all material for 48 h at 72 °C in a drying oven before weighing samples.

2.4.2.4. *Eurybia divaricata, Maianthemum racemosum* and *Polygonatum pubescens*

In mid-September we collected density, height, and reproductive status data on *E. divaricata* in the ten permanent 1m^2 quadrats in each open and fenced plot at six sites. If *E. divaricata* was not present in the initial quadrat location, we searched for the closest *E. divaricata*, and then placed the quadrat with the plant in one corner and shifted to maximize number of plants in the re-established quadrat. Thus, we recorded frequency and cover in the initial quadrats, and plant vigor in both the initial and additional quadrats.

In mid-June 2012 we recorded frequency, height, leaf width and reproductive status of *M. racemosum* and *P. pubescens* in multiple 2-m wide x 30m long randomly located transects at two sites that had >20 stems of either species (**Table 3**). Few or no plants were recorded in the

open plot transects: we therefore established additional transects near the open plots until ~ 10 stems were recorded of one of the species.

Table 3. Number of 2m x 30m transects and number of stems recorded for *Maianthemum racemosum* and *Polygonatum pubescens* June 2012.

Site	Location	No. transects	*Maianthemum* No. stems	*Polygonatum* No. stems
5	Fenced	4	22	27
	Open	8	9	11
8	Fenced	5	16	37
	Open	9	3	34

2.4.3. Earthworm monitoring

We assessed earthworm abundance in five randomly selected 0.25 m^2 quadrats in each open and fenced plot in late July 2008 – 2011. After removing all leaf litter from the quadrat, we extracted earthworms by pouring 3.79 l of aqueous mustard solution with a concentration of 15 g l^{-1} (Frontier Natural Products Co-op, Norway, IA) (**Fig. 5A**). This mustard solution causes a skin irritation resulting in rapid emergence of worms to the soil surface where they can be collected. We preserved specimens in 10% formalin and then weighed and identified each individual to species when possible (only sexually mature worms can reliably be identified to species).

We evaluated the effects of year (2008-2011), deer exclusion (open or fenced plot), plant invasion (dominated or not by non-native plant species), plant species richness, slug activity-density, leaf litter volume and vegetation height (see methods for these variables in section 2.4.1) on earthworm captures with GLMM with Poisson errors. We analyzed earthworm biomass separately with linear mixed models after log-transforming the data to meet the assumptions of normal distribution and constant variance. We used mixed models to account for the nested structure of the data. Therefore, site and plot within site were included as random factors in all models. We pooled data from five samples taken per plot each year to correct for lack of independence among samples. Models for each genus only included sites and years at which the genus was present throughout the study. In this case site was included as a fixed factor in the linear model.

2.4.4. Slug monitoring

We assessed slug activity-density at five randomly selected locations in each open and fenced plot in late July from 2008-2011. At each location we dug a 500 ml plastic container ≈ 3 cm into the ground. Each container had a lid and two holes cut into the sides (3cm in diameter) and was baited with beer as an attractant (**Fig. 5B**). The containers were established in such a way that holes were flush to the ground to allow easy slug access and were left open overnight. Slugs entered the traps during the night and drowned. We preserved specimens in 10% formalin and then weighed and identified each individual to species. Identification of 2008 samples was confirmed by the USDA National Malacology Specialist, Dr. David Robinson.

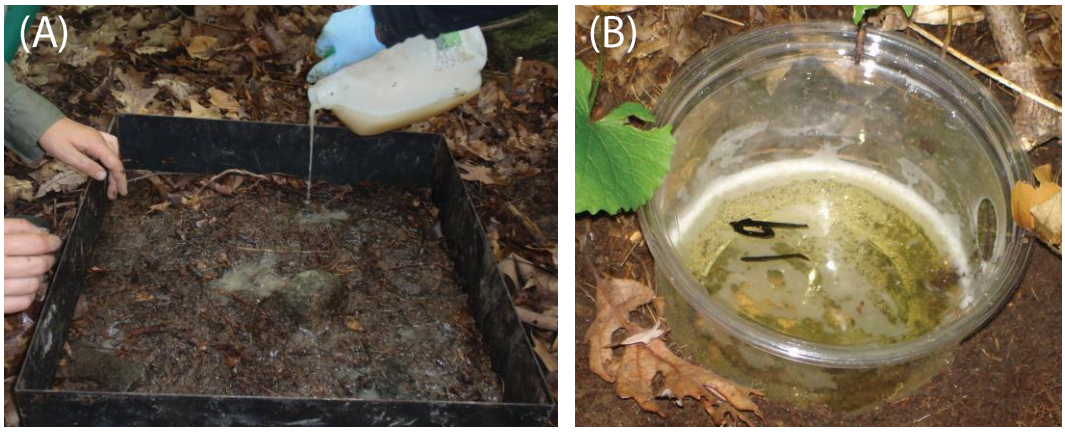

Fig. 5. Earthworm (A) and slug (B) monitoring at field sites at West Point NY. We sampled earthworms using an aqueous mustard solution (details in section 2.4.3) and slugs with modified pitfall traps baited with beer (details in section 2.4.4).

2.4.5. Root-weevil monitoring

We collected three 0.25 m^2 leaf litter samples in each open and fenced plot at all 12 sites 2009-2011. We sampled four times in 2009 (12/5, 1/6, 22/6 and 16/7) to determine the most suitable time to maximize capture rates. In the following years, we reduced litter sampling to two times in 2010 (21/4 and 18/5) and two times in 2011 (22/5 and 5/6). In each plot we used a square 0.25m^2 frame to delineate the sample unit, then removed all leaf litter and placed it into cotton pillowcases. All litter was transported to Cornell and placed in Berlese funnels with a 70% EtOH trap at the bottom. We left funnels in place for 7 days and then identified and counted the number of *B*. *pellucidus* captured in each trap. Leaf litter collected was oven dried for 72 h at 60°C and weighed. We used a generalized linear mixed effect model (GLMM) with binomial errors to test the effect of collection year, fencing and leaf litter biomass on *B. pellucidus* presence/absence. Using a subset of the data where *B. pellucidus* was present, we then tested the effect of the above factors on *B. pellucidus* relative abundance with a GLMM with Poisson errors. Both models included site and plot within site as random variables to account for lack of independence among samples. Only data from 2010 and 2011 were used for the analysis because in 2009 samples were taken adjacent to, but not within, fenced and open plots to minimize potential impact on plot vegetation and leaf litter. We found no impact and thereafter collected samples within the plots.

2.5. Selection of threatened or endangered (SAR) plant species

In 2008 we began the search for 4 SAR plant species to incorporate into our research. While numerous SAR occur at West Point, most are in unsuitable habitat and/or have very small populations. Our selection criteria consisted of: species present at West Point; state listed in NYS and/or nearby states; typical habitat sugar maple/red oak forest community, and population $\geq$ 100 individuals in a location near or on West Point with permission to work. We also desired to capture SAR in different plant families and their known or assumed response to deer herbivory or earthworm invasions. After one year of search, including the hiring of local expert botanists with knowledge of the species and their growing locations, we located populations of four SAR species that met our criteria: *Aristolochia serpentaria* L. (NYS Endangered in 2009,

and Threatened in 2012), *Agrimonia rostellata* Wallr. (NYS Threatened), *Carex retroflexa* Muhl. ex Willd (NYS Endangered in 2009 and Threatened in 2012), and *Trillium erectum* L. (state listed in nearby states, but not NY) (**Fig. 6**).

Fig. 6. Selected threatened or endangered species: (A) *Agrimonia rostellata,* (B) *Aristolochia serpentaria,* (C) *Carex retroflexa* and (D) *Trillium erectum.*

Agrimonia rostellata is a perennial herb 40-100 cm tall with compound leaves, each typically having 5-7 leaflets, arranged alternately on a stem sparsely covered with glandular hirsute hairs. Plants grow from a fibrous root with slender fusiform tubers. Flowers are produced in a raceme that may have one or more branches. Each raceme can have up to 30 flowers, and each fruit contains a single achene. Flowers are produced in mid-July and fruits develop August – September. Seeds germinate after two months of cold (2 – 5^{o} C) stratification. Plants typically produce an inflorescence during the second growing season. *Agrimonia rostellata* is a state Threatened species with 17 known populations in NYS. Deer herbivory is considered a potential threat to this species in NYS (New York Natural Heritage Program, 2013), and related *Agrimonia* species are readily consumed by deer (Atwood, 1941). We anticipated that deer would have a negative impact on this species, and had no *a priori* hypothesis for earthworm impact or threat by the other stressors under investigation.

Aristolochia serpentaria is a perennial herb with 6-12 cm long leaves arranged alternately on a stem up to 60 cm tall. Flowers are produced on short stems at or just above the soil, often hidden under leaf litter. Each flower can produce a single multi-seeded fruit capsule. Plants flower mid-May to July, and fruits are produced mid-July through September. *Aristolochia serpentaria* is a NYS Threatened species with at least 6 known populations in NYS. Very few studies have been conducted on this species, and none on ecology, demography or natural history. We anticipated that deer would have a negative impact and that earthworms would have a negative impact on seed production by removing leaf litter that conceals flowers.

Carex retroflexa is a cespitose sedge with narrow leaves 1.4-3 mm wide. Fruiting culms are 10-75 cm tall, and fruits are arranged in small star-like spikes along the upper portion of the culm. Plants flower May - June and fruits are produced June-July. *Carex retroflexa* is a state Threatened species with 13-35 known populations in NYS. Deer and earthworms are considered to benefit related *Carex* species (Hale et. al., 2006; Powers and Nagel, 2008; Fisichelli et. al., 2013) and we anticipated that both would independently and interactively have a positive impact on *C. retroflexa*.

Trillium erectum is a long-lived perennial herb with three leaves and a single flower at the top of a 20-40 cm tall stem. Plants grow from rhizomes, and a new stem is produced annually. Typically each rhizome produces a single stem but some rhizomes can have two or more stems in a single year. The stimulus to produce multiple stems is not known but may be associated with age or resource availability. Plants flower in May and produce a single multi-seeded fruit in July-August. Seeds germinate after 2 or more years of cold stratification, producing a cotyledon that remains present throughout the summer. The first true leaf is produced the following year, and after one or more years a single three-leaf stem appears. The first flower typically appears 5-7 years after seed germination. *Trillium erectum* is Exploitably Vulnerable in NYS and a state Threatened species in RI. Deer herbivory is cited as a major threat to related *Trillium* species (Anderson and Katz, 1993; Knight et. al., 2009) and we anticipated that deer would have a strong negative impact on *T. erectum*. Earthworms have both positive and negative impacts on other perennial herbs in the Liliaceae and we anticipated a negative impact on *T. erectum*.

2.6. Seeding experiment

To assess SAR germination rates under field conditions, we planted fresh seeds of three of the four SAR in 2009 and 2010, and monitored germination and survival at monthly intervals 2010 – 2012. We collected *Trillium erectum* seeds at West Point 6 August and planted seeds 16-18 August 2009. We purchased *A. serpentaria* seeds from Loess Roots (Stanton, NE), received the seeds 1 October and planted the seeds 4-6 October 2009. We collected *C. retroflexa* seeds at Wildcat Mountain State Forest and Clearwater Park, New Paltz, NY, 18 June, and planted seeds 10-11 August 2010. For all three species, we individually planted 20 seeds in each of four 20 x 20 cm permanently marked quadrats in each open and fenced plot (20 seeds/subplot, 4 subplots/plot, 24 plots, total 1920 seeds of each species). We did not plant seeds of the fourth SAR, *A. rostellata*, as we could not obtain sufficient numbers of seeds.

At each planting location, we carefully removed leaf litter before individually planting seeds with tweezers ~2 mm deep (*C. retroflexa*) or 5-8 mm deep (*A. serpentaria* and *T. erectum*) in soil, ~ 4 cm apart. We then lightly tamped the soil and replaced the leaf litter. We planted additional seeds of all three species in five reference germination flats (40 seeds each for *T. erectum*, 20 seeds each for *A. serpentaria* and *C. retroflexa,* plus 246 wild-collected seeds

of *A. rostellata)* kept in shaded cages to assess germination rates under common garden conditions and in the absence of competition or predation. We monitored seedling emergence and survival of all species beginning April 2010 through August 2012 at biweekly (2010) or monthly (2011-2012) intervals. *Aristolochia serpentaria* germinated in 2010, and *T. erectum* and *C. retroflexa* in 2011. We individually marked each germinated seedling using colored wooden markers and monitored survival and herbivory through late August 2012. In late August 2011 and 2012 we also recorded size (height and number of leaves or culms) of all surviving *A. serpentaria* and *C. retroflexa* (we did not record size of *T. erectum* seedlings as the majority was already dormant).

2.7. Transplant experiment

2.7.1. Propagation

We propagated seedlings of all four SAR (*C. retroflexa, A. rostellata, A. serpentaria* and *T. erectum*) to use in transplant experiments and in common garden mesocosm experiments (see Section 2.9). Due to the inability to collect sufficient seeds in the field, we worked with Dr. Joyce van Eyck of Boyce Thompson Institute, Ithaca NY to develop sufficient plant material through *in vitro* micropropagation techniques for all four species. The advantage of this approach was that we had genetically identical plants to test in different stressor treatments. Unfortunately, these efforts were successful only with *A. rostellata* (see below). Efforts to propagate *C. retroflexa* via tissue culture failed due to abundance of endophytes within leaf blades, which led to fungal contamination of most propagated seedlings. Efforts to propagate *A. serpentaria* were initially successful using leaf tissue collected in May from commercially purchased plants grown at Cornell University, but were unsuccessful using leaf tissue collected in July from local wild plants growing at West Point. We therefore transplanted 11 *A. serpentaria* from the West Point population and maintained them in outdoor and then greenhouse conditions through the following spring, to obtain fresh leaf material the following May; however, plants failed to thrive and this effort was unsuccessful. Dr. Van Eck successfully propagated *T. erectum* using dormant buds collected in August from the Bobolink Hill population (site of demographic monitoring plots; see section 2.8). We collected 11 *T. erectum* from the West Point population in September and initiated tissue culture. However, plantlets developed from only a few of the *T. erectum* rhizomes, and grew too slowly to feasibly obtain an adequate number of seedlings for use in experiments.

Consequently, we grew *C. retroflexa*, *A. serpentaria*, and *T. erectum* from seeds, and propagated *A. rostellata* using tissue culture, as described below. This created an unfortunate delay in our ability to transplant seedlings to our field sites, and consequently we were able to follow the fate of our transplanted individuals for only two growing seasons, which in turn affected our ability to estimate transition probabilities.

2.7.1.1. *Agrimonia rostellata*

We collected 15 *A. rostellata* plants from the population at Blue Lake, Sterling Forest State Park, Tuxedo, NY, transferred plants to 15 cm diameter pots, and maintained these plants under greenhouse conditions in the Department of Natural Resources at Cornell University. Dr. Joyce Van Eyck of Boyce Thomson Institute, Ithaca, NY, developed an *in vitro* micropropagation protocol for *A. rostellata* using these plants.

To establish *in vitro* material, we harvested nodal sections from each plant and removed the leaves. The nodal cuttings were washed thoroughly in a solution that contained a small amount of either Alconox (Alconox, Inc. New York, NY, www.alconox.com) or an antibacterial hand soap. The material was then disinfected using a combination of washes in a detergent solution, 70% ethanol, bleach, plus vacuum infiltration with a solution of the antifungal compound, Miconazole, and its inclusion at 20 mg/l in the culture medium. A Murashige and Skoog-based medium supplemented with 0.1 mg/l indole-3-butyric acid, 5 g/l charcoal, and 7 g/l Sigma agar was found to be best for multiplication of shoots and root formation. A starting population of 39 contaminant-free, *in vitro* plants, representing 7 maternal lines, were scaled up to 1013 plants in 4 months. Cultures were maintained in test tubes (25 x 150 mm) with transparent plastic caps, which were secured to the tubes with a small piece of Nescofilm (Karlan Research Products, Santa Rosa, CA www.karlan.com). Each tube contained 15 ml of medium. Cultures were kept under lights at (light intensity: average of 65 uE $m^{-2}s^{-1}$) on a 16 h light/8 h dark photoperiod at 25C $\pm$ 2C.

We divided plants every 4 weeks and transferred to fresh medium. Divisions were made by removing the plant from a test tube, placing it on a sterile paper towel then using a scalpel to make a vertical cut through the base of the shoot between individual shoots and the associated roots. Each plant was then transferred to an individual tube containing the appropriate medium (**Fig. 7**).

Fig. 7. *In vitro* propagation of *A. rostellata*.

Four to five weeks after transfer of cuttings to medium for rooting, well-rooted plants were removed from test tubes, and the culture medium was washed from the roots in tepid water. Large plants with multiple stems were divided using a scalpel, and the freshly cut plantlets individually transferred to potting soil. Plants were transferred to multi-insert trays of a soil mix that contained the following components: 538.6 g Unimix + III (Griffin Greenhouse and Nursery Supplies, Tewksbury, MA), 2.27 kg lime (Hummert International, Earth City, MO), 0.161 m^3 peat moss (Hummert International), 0.34 m^3 vermiculite (Hummert International) and 2.27 kg Osmocote 17-7-12 (Grower Supply Inc., Forest Hill, LA)]. Trays containing plants were covered with plastic domes fitted for the trays to maintain a humid environment for gradual acclimation to

ex vitro conditions, and placed in a growth chamber (21.0 C, 85% R.H., 13-hour photoperiod). After 3 days, the plastic domes were lifted slightly and then completely removed after a total of 7 days from the transplant date. Plants were fertilized with Miracle-gro liquid fertilizer ® at half-strength (10 drops/liter). Plants were transferred to a greenhouse for 7 days, then transferred to outside shade-cages to harden off for an additional 14 days, before transplanting into experimental mesocosms and field sites.

2.7.1.2. *Aristolochia serpentaria*

We planted 2000 *A. serpentaria* seeds (purchased from Loess Roots, PO Box 877, Stanton, NE 68779) 12 October 2010 in small flats, and placed flats in a temperature-controlled cold room. We transferred flats to a greenhouse 16 February 2011. The first germinants were observed 29 March 2011, and seedlings were transferred into individual cells (3.8 x 3.8 x 6 cm) 30 April 2011. On 4 May 2011 we measured leaf width and counted number of leaves of each *A. serpentaria* to be planted in experimental mesocosms and at West Point (see section 3.7) and transplanted seedlings 10-13 May 2011, respectively.

2.7.1.3. *Carex retroflexa*

We collected *C. retroflexa* seed 16 June 2010 from 64 individual plants (60 at Clearwater Park, New Paltz, NY and 4 at Wildcat Mountain State Forest). While growing plants from seed, we tested if germination rates differed between maternal lines within and between populations (West Point seedlings) and tested if germination rates differed by stratification treatment (common garden seedlings).

2.7.1.3.1. West Point seedlings

On 22 June 2010 we counted seeds from each maternal line into groups of 20 (maximum 5 groups/maternal line), placed each group of seeds into an individual gauze bag, and sewed the bag shut. We placed the gauze bags under leaves in an outdoor mesocosm at Cornell University, Ithaca, NY on 29 July 2010 for 33 days of warm moist stratification. On 31 August 2010 we transferred the gauze bags to plastic shoeboxes filled with moist sand, which we placed into an unlit cold-room (constant 3-5 °C) for 140 days of cold moist stratification. We then opened each gauze bag, and transferred each bag of 20 seeds to an individual petri dish with moist soil-free potting mix, which we placed into an incubator at 10/30 °C 12/12 day/night 18-20 January 2011. After 3 weeks we transferred germinated seedlings into individual cells (3.8 x 3.8 x 6 cm) filled with a soil-free potting mix, keeping track of maternal line for each plant, and then maintained seedlings in a greenhouse. We measured all seedlings (number of culms and maximum culm height) and then transferred seedlings to outside shade-cages to acclimate for 7 days before transplanting into field sites.

2.7.1.3.2. Common garden seedlings

After removing seeds for West Point seedling propagation (see above), we mixed all maternal lines for remaining seeds collected from New Paltz and Clearwater, and separately mixed *C. radiata* seeds collected from Richford NY, and tested if 1) germination percentage varies by stratification treatment (warm moist followed by cold moist, compared to only cold moist

stratification) and 2) if germination requirements differed between the rare *C. retroflexa* and the common *C. radiata*. For each collection locale we counted seeds into groups of 50, placed each group of seeds into an individual gauze bag, and sewed the bag shut. Ten bags of each collection locale were placed under leaves in an outdoor mesocosm at Cornell University, Ithaca, NY on 16 July 2010 for 31 days of warm moist stratification, and ten bags were placed in a room with constant 21 °C for the same time period. On 16 August 2010 all bags were then randomly placed in 5 shoeboxes (12 bags/box) filled with moist sand, and placed in an unlit cold room (constant 2-5 °C) for 155 days cold moist stratification. We conducted a preliminary test to determine efficacy of different planting media (agar, soil-free potting mix, sand, moist paper towel) and determined that soil-free potting mix provided the optimal germination environment. We then opened each gauze bag, and transferred each set of 50 seeds to an individual petri dish with moist soil-free potting mix, which we placed into an incubator at 10/30 °C 12/12 day/night 13-14 January 2011. After 3 weeks we transferred seedlings into individual cells (3.8 x 3.8 x 6 cm) filled with a soil-free potting mix, keeping track of collection locale for each plant, and then maintained seedlings in a greenhouse. We measured all seedlings (number of culms and maximum culm height), then transferred seedlings to outside shade-cages to acclimate for 7 days before transplanting into experimental mesocosms.

2.7.1.4. *Trillium erectum*

In August 2007 we collected 23 large ripe *T. erectum* fruits from three forests near Ithaca, NY. We individually opened each fruit, removed the seeds and washed off the fruit pulp, then placed seeds in small trays, one to two trays/fruit depending on number of seeds. We placed all small trays into two large flats, and kept flats outdoors in screened cages under a forest canopy to emulate more 'natural' forest conditions (temperature, precipitation and light) typically encountered by seeds remaining in the field. We moved flats in cages into a 1m deep north-facing coldframe each November/December after the soil was frozen, and removed them in April once nighttime temperatures remained above freezing. We added supplemental water only if trays were obviously dry and no rain had fallen for >2 weeks. We recorded germination monthly 2008-2012. Cotyledons emerged spring 2009, and became one-leaf seedlings spring 2010. On 18-20 August 2010 we transplanted rhizomes into individual cells (3.8 x 3.8 x 6 cm) filled with a soil-free potting mix, keeping track of maternal line of each seedling. We randomly placed all seedlings into flats, and returned flats to the outdoor cages. We tamped the soil and seeds remaining in each of the germination trays and returned the trays to the outdoor cages. All trays and flats were transferred to the cold frame in November. On 14 March 2011 we transferred the trays containing the dormant transplanted seedlings to an indoor unlit space and gradually raised the temperature from 7 to 18 °C over a 7d period. We then placed the trays under grow lights and watered as needed while seedlings emerged. On 10 April we measured leaf width of each trillium to be planted in experimental mesocosms (see section 2.9) and transplanted seedlings 15 April 2011. On 10 April we measured leaf width of each *T. erectum* to be planted in experimental mesocosms (see 2.9.3) and transplanted seedlings 15 April 2011. On 20 April we measured leaf width of each *T. erectum* to be planted at West Point (see 2.9), and transplanted seedlings 27-29 April 2011.

2.7.2. Transplant and data collection

We transplanted seedlings of all four SAR (*C. retroflexa, A. rostellata, A. serpentaria* and *T. erectum*) into each open and fenced plot at West Point in May 2011 (20/plot, 24 plots, total 480

seedlings/species), keeping track of maternal line for *A. rostellata* and *T. erectum*, and population for *C.* retroflexa; we raised seedlings of *A. serpentaria* from commercial seed without maternal line information. We grew *C. retroflexa* seedlings from seed and seedlings were ~ 4 mo. old at the time of planting. We grew *A. serpentaria* seedlings from commercial seed without maternal line information, and seedlings were ~ 3 mo. old at the time of planting. We propagated *A. rostellata* seedlings using tissue-culture techniques (see section 2.7.1.1) and planted them into individual cells 9 April 2011. We grew *T. erectum* seedlings from seed collected near Ithaca NY August 2008; seedlings were almost 3 years old with a single leaf at the time of planting (this species germinates 2 years after planting, and produces the first true leaf the following year).

Before transplanting, we took initial measurements for each seedling (leaf width for *T. erectum*, height and culm number for *C. retroflexa*, and height and leaf width for *A. serpentaria* and *A. rostellata*). Four seedlings (one/SAR) were planted within a 30-40 cm diameter area, at 20 randomly located positions/plot. This design allowed us to test the effect of deer exclusion (open and fenced plots), slug exclusion, nutrient addition and their interactions on the survival and fitness of SAR seedlings. Maternal line was also randomly distributed across treatments, such that each treatment received as many maternal lines as possible. We applied a molluscicide (Earth-tone, iron phosphate pellets) at the time of planting, and at monthly intervals for slug exclusion. We experimented with various methods to exclude slugs from experimental plants. Unfortunately, none of the advertised or widely described methods to exclude slugs showed any potential to exclude slugs from experimental seedlings when tested experimentally. This included tangle foot and copper barriers, as well as diatomaceous earth and other barriers. Ultimately, we needed to apply molluscicide despite the known effects on non-target organisms including invertebrates and earthworms. However, these effects are strictly localized to the treatment areas, and we do not expect them to affect other results in our treatments. We applied slow release fertilizer (Osmocote) at the time of planting for nutrient addition, and again in spring 2011.

Before transplanting we confirmed that initial plant size (height, leaf width or leaf length, depending on the species) did not differ among treatments. We compared plant size with a LM with size variable as response and treatment assignment as response. We recorded survival, growth, and herbivory at biweekly to monthly intervals 2011 – 2012, and recorded size of each surviving individual in late August each year, using the same measures employed prior to planting. We also recorded reproductive effort for *C. retroflexa* (number flowering culms, and total seed production) and *A. rostellata* (inflorescence length and total flower production). Before testing for the effect of treatment on each SAR species, we evaluated the effect of maternal line on seedling survival, growth and reproduction.

2.8. Plant mark-recapture

In 2009 we permanently marked 80-170 individuals of each species (**Table 4**) (based on population size) and erected deer fences encompassing half of the marked individuals in late spring (*T. erectum*) and early summer (*A. serpentaria, A. rostellata* and *C. retroflexa*). We collected demographic data at biweekly to monthly intervals through the entire growing season (April – October) 2009-2012. We recorded presence/absence, stem number, stem height, flower and fruit presence, and evidence of browse, for each individual plant. In addition, we measured leaf width for *T. erectum* and *A. serpentaria*, counted number of leaves for *A. serpentaria* and *A. rostellata,* and number of culms *for C. retroflexa*. We also recorded seed production for all or a subset of individuals of each species.

Table 4. Species, initial number of marked individuals, location and fence construction date for SAR plant species selected for demographic monitoring.

Species	No. marked Plants	Location	Fence constructed
Agrimonia rostellata	100	Blue Lake, Sterling Forest SP, NY	16 July 2009
Aristolochia serpentaria	107	West Point, NY	15 July 2009
Carex retroflexa	80	Clearwater Park, New Paltz, NY	15 July 2009
Trillium erectum	170	Bobolink Hill, Richford, NY	20 May 2009

2.9. Common garden experiments

2.9.1. Slug palatability tests

2.9.1.1. Slug feeding trials

During 2008, we tested the feeding preferences of four slug species (native: *Deroceras laeve* and *Philomycus* sp, non-native: *Arion subfuscus* and *Limax* maximus) to assess feeding impact on native and non-native seedlings and fruits (**Table 5**). These slug species are the most common we encountered in our field surveys at West Point. We initially anticipated testing *Deroceras reticulatum,* the common garden slug. However, this species is rarely encountered in forest interiors and we did not observe it in our West Point surveys. For most trials, we tested 3-5 different individuals within each slug species. Replication depended on availability of plant material. We placed one slug individual into a 500 ml plastic container covered with a moistened paper towel to maintain humidity (**Fig. 8A-B**). We then placed the same amount of plant material into each terrarium and recorded consumption as percentage disappearance of tissue daily for 4d. We starved slugs for 48 h prior to the start of the experiment. At termination of the trial, we planted seeds/fruits exposed to slugs and unexposed control seeds/fruits, and monitored germination rates from 2009-2012 to determine if slug feeding affected germination.

Fig. 8. Terrarium setup for slug feeding trials (A, B, section 2.9.1.1) and for *T. erectum and T. grandifolium* consumption trial (section 2.9.1.2). Picture shows consumption of *T. erectum* fruit after 5d of exposure (C).

Table 5. Plant species, species abbreviation and plant tissue offered to slug species of native and non-native origin (native: *Deroceras laeve* and *Philomycus* sp, non-native: *Arion subfuscus* and *Limax* maximus).

Plant species	Abbreviation	Tissue
Actaea alba	Act alb	Fruit
Actaea rubra	Act rub	Fruit
Ageratina altissima	Age alt	Seedling
Alliaria petiolata	All pet	Seedling
Aquilegia canadensis	Aqu can	Seedling
Aralia racemosa	Ara rac	Fruit, Seedling
Arisaema triphyllum	Ari tri	Fruit, Seedling
Berberis thunbergii	Ber thu	Fruit
Brachyelytrum erectum	Bra ere	Seedling
Bromus latiglumis	Bro lat	Seedling
Campanula americana	Cam ame	Seedling
Carex radiata	Car rad	Seedling
Caulophylum thalictroides	Cau tha	Fruit
Circaea lutetiana	Cir lut	Seedling
Clintonia borealis	Cli bor	Fruit
Crataegus sp	Cra sp	Fruit
Cryptotaenia canadensis	Cry can	Seedling
Elymus hystrix	Ely hys	Seedling
Fraxinus americana	Fra ame	Fruit
Geranium maculatum	Ger mac	Seedling
Geum canadense	Geu can	Seedling
Ilex verticillata	Ile ver	Fruit
Lonicera sp	Lon sp	Fruit, Seedling
Maianthemum canadensis	Mai can	Fruit
Maianthemum racemosum	Mai rac	Fruit, Seedling
Medeola virginiana	Med vir	Fruit
Microstegium vimineum	Mic vim	Seedling
Mitchella repens	Mit rep	Fruit
Nyssa sylvatica	Nys syl	Fruit
Panax quinquefolius	Pan qui	Seedling
Podophyllum peltatum	Pod pel	Fruit
Polygonatum pubescens	Pol pub	Fruit, Seedling
Prunus serotina	Pru ser	Fruit
Rhamnus cathartica	Rha cat	Fruit, Seedling
Rosa multiflora	Ros mul	Fruit
Sambucus canadensis	Sam can	Fruit, Seedling
Sanguinaria canadensis	San can	Seedling
Sanicula gregaria	San gre	Seedling
Streptopus roseus	Str ros	Fruit
Trillium erectum	Tri ere	Seedling, eliasome
Trillium grandiflorum	Tri gra	Eliasome
Trillium undulatum	Tri und	Eliasome
Viburnum acerifolium	Vib ace	Fruit
Viburnum lantanoides	Vib lan	Fruit

We ran separate LMs for each plant tissue (seedling, fruit and eliasome) to evaluate the effect of slug and plant species origin on percent tissue consumed by slugs. In all models, slug species were nested within slug origin and plant species were nested within plant origin. Consumption was arcsine-root square transformed. We did not test for interactions, as we did not have enough degrees of freedom to adequately fit a full model.

2.9.1.2. *Trillium erectum* and *T. grandiflorum* slug preference and germination trial

We tested the effect of slug feeding (*Arion subfuscus*) on fruits and seed of two congeners: SAR *T. erectum* and common *T. grandiflorum,* on 24 to 28 July 2011. We placed one full fruit or one seed (with eliasome) of each species into an indoor terrarium (8 cm in diameter) lined with 2 cm of moistened flower foam. We randomly assigned terrariums to slug (one *A. subfuscus* added) or control treatments (20 replicates/ treatment/*Trillium* species/*Trillium* organ for a total of 120 terrariums) (**Fig. 8**). We starved slugs for 48 h prior to the start of the experiment and weighed them before placing them in the terrarium in order to control for the effect of slug size on consumption.

We recorded percent consumption as percentage disappearance of tissue (due to slug feeding and/or tissue decomposition) daily and terminated the experiment after 5 d when the majority of seed had been consumed or disappeared. After termination of the experiment we planted all fruit into individual pots to test whether *A. subfuscus* feeding affected germination. We assessed *Trillium* germination on 6 May 2013. We evaluated the effect of time (ordered factor), slug treatment, plant species and plant organ on slug consumption with a LMM. Terrarium identification was included as a random factor, to account for lack of independence among samples taken from the same terrarium on consecutive days. We tested for differences in germination rate according to slug treatment with a GLM with binomial errors.

2.9.1.3. Feeding trial and molluscicide effectiveness

Coincident with our field study of slug impact on transplanted seedlings of the four SAR (see section 2.7) we tested the effectiveness of molluscicide (1% iron phosphate) for preventing slug herbivory on the SAR and on 13 native and non-native plant species in June 2011. Two of the species tested were common and closely related to two of the SAR species (*C. retroflexa* and *C. radiata*; *A. rostellata* and *Agrimonia gryposepela*) to see if preferential slug herbivory may be a factor in rarity of these species in comparison to their common congeners. We also tested two growth stages for SAR *T. erectum* (cotyledon and one-leaf) and two propagation methods for SAR *A. rostellata* (wild-collected seed and tissue culture).

We transplanted seedlings into individual outdoor terrariums (20 by 20 cm) randomly assigned to one of three treatments (control, one *A. subfuscus* or one *A. subfuscus* plus molluscicide) in six blocks (15 taxa x 3 treatments x 6 replicates = 270 terrariums). Slugs were held for 48 hours without food prior to placement in the terrariums. Seedlings were transplanted into individual terrariums and molluscicide was added per manufacturer's instructions (Earth-tone® Slug & Snail Control, The Espoma Co. Millville NJ), prior to introduction of slugs. Despite our efforts to prevent slug escape, we were unable to locate 13% of the slugs at the end of the experiment, with 78% (18 of 23 slugs) missing from the slug present–no molluscicide treatment. We assumed that all missing slugs escaped from the terrarium because we did not find any

indication of slug activity in those compartments. Missing a dead slug is nearly impossible in a terrarium of small size (smell is pungent and slugs secrete considerable amount of slime from time of molluscicide consumption until death occurs up to 12 h later). Given the disproportionate amount of missing slugs from the control treatment, we used a subset of the data for which we had encountered the slug (live or dead) at the end of the experiment for all analyses. Death encounters included dead bodies and instances where we smelled the decaying body and could see slug slime in the terrarium. To evaluate the overall effectiveness of the molluscicide for preventing slug herbivory, we compared the probability of plant consumption according to treatment and plant species with a generalized linear model with binomial errors.

2.9.2. Earthworm effects on plant germination

In spring 2008 we established a factorial experiment to assess impact of single and multiple earthworm species on germination and seedling survival of native forest species (Hypothesis 1: Earthworms reduce germination of native forest plant species; Hypothesis 2: Earthworms reduce seedling survival and juvenile growth of native forest plant species).

We established 100 mesocosms in spring 2008, each consisting of a 120 L plastic tree-pot (diameter 60 cm, 50 cm tall) filled with 5 cm of fine-textured playground sand at the bottom and commercial potting soil, placed inside a mesh 'cage' 75 cm x 75 cm x 120 cm: (Reptarium cage), which reduces light levels by ≈ 60%. We installed an additional shade cloth roof to further reduce light levels and temperatures in the mesocosms in July 2009. At initial set-up, each tree-pot received 400 g of dried leaf litter (equivalent of 4 years of leaf fall) collected from a non-earthworm-invaded forest. To establish a natural microbial community, each pot was inoculated with 1-L of filtered (35 micron) non-earthworm-invaded forest soil water. To simulate annual leaf input, we added freshly collected and air-dried leaves in November/December each year to each mesocosm: in November 2008 we added 35 g *Acer saccharum,*35 g *Fraxinus americana,* and 10 g *Quercus rubra*; on 16 December 2009 and 11 November 2010 we added 60 g *A. saccharum*, 30 g *F. americana*, 10 g *Q. rubra*). To simulate annual snowfall we collected fresh snow from the top of each mesocosm in winter and placed it inside the mesocosm ~10 cm deep.

We arranged mesocosms in double-rows of 25 pots, with half the pots facing north and half south. We placed additional (unused) mesocosms on the east and west ends of each row, to ensure comparable light and temperature conditions in all test mesocosms (**Fig. 9**). We randomly assigned mesocosms to one of five earthworm treatments (*Lumbricus terrestris* L., *Lumbricus rubellus* Hoffmeister*, Amynthas spp.*, "all worms" or "no worms" [Control]). All treatments were evenly and randomly distributed between north and south-facing mesocosms.

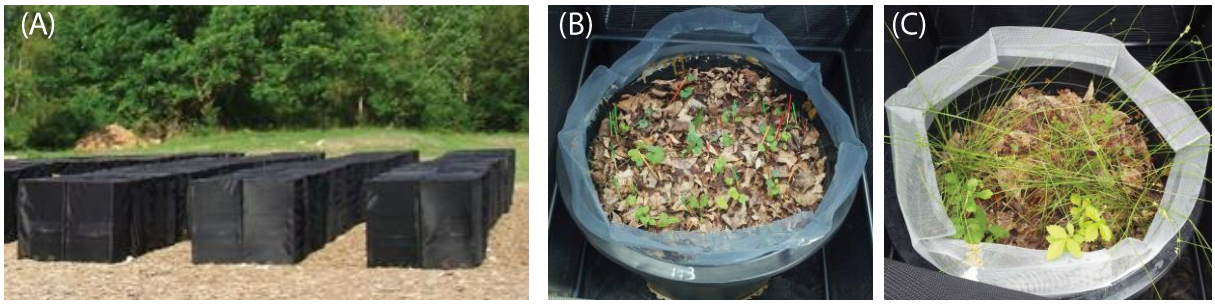

Fig. 9. Outdoor mesocosm layout (A), close up of established mesocosm showing germinants (B, section 2.9.2) and close up of establish mesocosm showing transplanted SAR (C, section 2.9.3).

We stocked mesocosms in June and July 2008 based on reported field densities in the Ithaca, NY area. We collected *L. terrestris* (6 mature individuals/pot) in a local agricultural field; and purchased *L. rubellus* (20 reproductive individuals/pot) from a supplier in OH). We collected sexually immature *Amynthas spp.* (15 individuals/pot) locally at Cornell Plantations. Individuals of this last genus can easily be identified by their ring of bristles around each segment and their unique wriggling behavior which sets them apart from any other earthworm represented in our area. We sent a local worm sample for identification to Dr. Bruce A. Snyder, Division of Biology, Kansas State University, Manhattan, KS 66506-4901, who identified the individuals as a mixture of *A. agrestis* and *A. hilgendorfi*. We also created mixed treatments using 2 *L. terrestris*, 8 *L. rubellus*, and 7 *Amynthas* spp./pot. For each species we weighed 10-20 individuals to obtain an average weight, and then multiplied this weight by the number of earthworms/treatment to establish a target group weight. We then selected earthworms of comparable size, weighed each group, and replaced individual earthworms as needed to remain within 5% of the target weight. One earthworm species (*L. rubellus*) was accidentally introduced into the 20 control mesocosms in late July 2008. Over half the earthworms were extracted by repeated electroshocking; however, earthworms remained in some pots. We therefore established 20 Replacement Control mesocosms in August 2008, all facing north due to logistical limitations. The leaf litter (and thus microbial tea) used in the replacement control mesocosms was collected from the same non-earthworm invaded forest, but differed from the leaf litter used in the original 100 mesocosms. We assessed earthworm survival in additional mesocosms of the same characteristics as the ones used for the experiment. We wanted to assess overwinter survival of the three earthworm species in our mesocosms, without disturbing the actual experimental mesocosms. We therefore created additional mesocosms and stocked them with earthworms to assess survival, and noticed earthworm winter mortality. Therefore, we re-stocked earthworms in June each year and in 2009-2011 and we increased the 2008 stocking rate by 50% for both *Amynthas* spp. (to 23 worms/mesocosm) and *L. rubellus* (30 worms/mesocosm) to provide a more pronounced worm impact. In all three years we collected *Amynthas* spp. and *L. terrestris* near Ithaca NY and purchased *L. rubellus* from Bob Lytle in Columbus, OH.

We began adding ripe seeds to our mesocosms in summer 2008. By the end of 2010 we had added a total of 19 species and 425,580 seeds (**Table 6**). The number of seeds added to the mesocosms varied by species, largely driven by seed availability and seed size. For three species we had <4,000 total seeds available and we therefore placed them into a subset of the mesocosms (10 Original Controls, 10 Replacement Controls, and 10 All Worms). We monitored seedling emergence at weekly (2008), biweekly (2009) intervals, or monthly intervals

throughout the 2011 growing season. We visually estimated percent cover of leaf litter and bare soil, and recorded evidence of earthworm presence (middens, castings) and activity.

Table 6. Plant species, status, life form, number of seeds/mesocosm and date (MM/DD/YYYY) seeds were added to mesocosms.

Species	Family	Origin	Life form	N seeds planted	Date planted
*Actaea alba**	Ranunculaceae	Native	Forb	40	9/8/2008
Agrimonia gryposepela	Rosaceae	Native	Forb	50	10/15/2009
Alliaria petiolata	Brassicaceae	Native	Forb	500	8/12/2009
Allium tricoccum	Alliaceae	Native	Forb	169	9/18/2008
Aquilegia canadensis	Ranunculaceae	Native	Forb	555	7/18/2008
Aristolochia serpentaria	Aristolociaceae	Native	Forb	50	10/18/2010
Berberis thunbergii	Berberidaceae	Introduced	Woody	200	11/9/2009
Brachyelytrum erectum	Poaceae	Native	Graminoid	59	9/9/2008
Carex radiata	Cyperaceae	Native	Graminoid	1000	7/16/2008
*Caulophyllum thalictroides**	Berberidaceae	Native	Forb	131	9/8/2008
Geranium maculatum	Geraniaceae	Native	Forb	58	7/2&9/2008
Geranium maculatum	Geraniaceae	Native	Forb	44	7/1/2009
Phryma leptostachya	Phyrmaceae	Native	Forb	150	10/12/2009
*Polygonatum pubescens**	Liliaceae	Native	Forb	23	9/8/2008
Ranunculus recurvatus	Ranunculaceae	Native	Forb	200	6/16/2009
Sanguinaria canadensis	Papaveraceae	Native	Forb	54	6/27/2008
Sanguinaria canadensis	Papaveraceae	Native	Forb	25	6/26/2009
Thalictrum dioicum	Ranunculaceae	Native	Forb	100	6/21/2009
Tiarella cordifolia	Saxifragaceae	Native	Forb	200	6/29/2009
Trillium erectum	Liliaceae	Native	Forb	140	8/7/2008
Trillium erectum	Liliaceae	Native	Forb	305	8/5/2009
Viburnum lantanoides	Adoxaceae	Native	Woody	145	9/9/2008

*= number of fruits.

We marked and followed the fates of 10 or 20 individuals of six species (**Table 7**) to assess seedling survival and growth under different earthworm treatments. At the end of the monitoring period we removed all surviving seedlings from each mesocosm, oven-dried the material for 48 h, then recorded the combined weight of plants from each mesocosm.

In August 2011 we unexpectedly discovered earthworms in our earthworm-free replacement control mesocosms. We assessed all control pots for evidence of earthworm presence (castings) and determined that at least 40% were invaded.

Table 7. Species, year and number of individuals monitored for survival and growth.

Species	Year	Survival Period	# Seedlings
Aristolochia serpentaria	2011	Growing season	20
Alliaria petiolata	2010, 2011	Growing season	10
Aquilegia canadensis	2008-09	Overwinter	10
Berberis thunbergii	2011	Growing season	10
Trillium erectum *	2011	Growing season	10
Viburnum lantanoides	2010	Growing season	10

** = only survival recorded*

In September 2011 we terminated the experiment: We removed all remaining leaf litter from all mesocosms and recorded the remaining dry leaf biomass in each mesocosm. We subsequently extracted earthworms using a mustard solution 3.79 l of mustard solution at 15 g l^{-1} (Frontier Natural Products Co-op, Norway, IA) and preserved worms in 10% formalin, then identified and weighed (wet mass) each individual. Earthworm extraction was conducted during a cold, rainy period and the number of earthworms removed was very low, relative to the number stocked in each mesocosm in spring 2011. We therefore hand-sorted for any remaining earthworms in the upper 20-25 cm of each mesocosm (due to logistical constraints we did not sort through the lowest 5-10 cm; we saw no evidence of earthworms in this lower layer). We also counted and removed all seedlings of germinated *Caulophyllum thalictroides*, a species that initially developed a very large root system without producing above-ground shoots.

At the end of the experiment we found earthworms in all treatments, including controls. Given that earthworms colonized experimental mesocosm we used leaf litter disappearance as a cumulative indicator of earthworm activity throughout the course of the study, rather than the original treatment assignments. Leaf litter disappearance is associated with earthworm invasion and often used as an indicator of their impacts (Suarez et. al., 2006; Holdsworth et. al., 2012). In our study, control (original and replacement) mesocosms had significantly higher leaf litter biomass remaining at the end of the four years than mesocosms with earthworms, but leaf litter biomass did not differ between treatments with different earthworm species. We obtained similar results regardless of whether we used initial earthworm treatments (not presented) or remaining leaf litter biomass at the end of the experiment.

The effect of treatment and aspect on the probability of seed germination was evaluated with independent GLM models (binomial errors) for each plant species. We used separate models for each plant species because species were planted according to seed availability, which resulted in different planting dates and different number of replicates or treatments for each plant species. Data for multiple years was summarized to avoid lack of independence among samples from the same mesocosms.

2.9.3. Root-weevil and earthworm effects on plant survival and growth

In spring 2010 we established a factorial experiment to assess impact of two earthworm species and a root weevil (*B. pellucidus*) on seedling survival and growth of four SAR and native forest species (Hypothesis 1: Earthworms and *B. pellucidus* reduce survival and juvenile growth of SAR plant species; Hypothesis 2: Earthworms and *B. pellucidus* effect on SAR increases when both organisms are present).

We established 120 mesocosms in spring 2009, following the same protocol as for the previous experiment (section 2.9.2, **Fig. 9**). We randomly assigned mesocosms to one of three earthworm treatments (*Lumbricus terrestris, Amynthas* spp. or No worms [Control]) and one of two weevil treatments (present or absent). All treatments were evenly distributed between North and South mesocosms.

We stocked earthworms in mesocosms August 2009 and 10 June 2010 (see section 2.9.2 for details) and in June 2010 we planted two native forest species known to be favored by *B. pellucidus* (to ensure food availability): two red oaks (*Quercus rubra*) and one black cherry (*Prunus serotina* Ehrh.) seedlings, and four *Q. rubra* acorns and two species with unknown palatability (one bottle-brush grass (*Elymus hystrix* L.) and one Solomon Seal (*Polygonatum pubescens*). All plants were grown from locally collected seed.

To simulate annual leaf input, we added 100g of locally collected and dried leaf litter (60 g *Acer saccharum*, 30 g *Fraxinus americana* L., 10 g *Q. rubra*) on 16 December 2009 and 11 November 2010, and 30 g (25 g *A. saccharum* and 5 g. *F. americana*) on 9 November 2011. To simulate annual snowfall we collected fresh snow from the top of each mesocosm in winter 2010 and 2011 and placed it inside the mesocosm ~10 cm deep. On 13 June 2011 we re-stocked earthworms at the same densities as in June 2010 and added three pairs of *B. pellucidus* collected in the Ithaca NY area.

We planted 3 seedlings of each SAR (two seedlings of *T. erectum*) in April-May 2011, keeping track of maternal line of each individual (see section 2.7.1). Before transplanting, we took initial measurements for each seedling (leaf width for *T. erectum*, height and culm number for *C. retroflexa*, and height and leaf width for *A. serpentaria* and *A. rostellata*). We recorded growth and survival of all SAR in September 2011 and at the end of the experiment in June 2012.

In early June 2012 we terminated the experiment. We recorded survival and size of each SAR individual, using the same measures employed prior to planting. We also recorded reproductive effort for *C. retroflexa* (number of flowering culms and total seed production) and *A. rostellata* (inflorescence length and total flower production). We then carefully removed each plant (SAR seedlings and native forest species) and washed the roots, then oven-dried each individual and separately recorded weights of above- and below-ground material. We removed all remaining leaf litter from each mesocosm and placed it in a Berlese Funnel for 7 d to extract *B. pellucidus* and then oven-dried and weighed the leaf litter. Lastly, we extracted earthworms using a mustard solution (3.79 l of solution at 15 g l^{-1} water; dry mustard obtained from Frontier Natural Products Co-op, Norway, IA). We preserved earthworms in 10% formalin, then identified and weighed (wet mass) each individual.

2.10. Statistical analyses

We analyzed data with a combination of Linear Models (LM), Linear Mixed Models (LMM) and Generalized Linear Models (GLM). LMMs included site and plot within site as random factors to account for data collected at consecutive times from the same plot (for example, earthworm monitoring, transplant and reseeding experiments). For the mark-recapture data we also included plant identity as a random factor. We used GLM with Poisson errors to analyze effects of selected factors on count response variables, such as for number of leaves or number of siliques; and GLM with binomial responses to evaluate effects on survival, presence/absence or probability of flowering. We examined and confirmed that test assumptions were met for all cases. When necessary, we log-transformed variables (unless it is stated otherwise) in order to meet model assumptions. Specific tests (beyond the LMM and GLMM models) are described at the end of the method section of each experiment.

We evaluated the explanatory power of competing models with Akaike Criterion corrected for small samples sizes (AICc) (Burnham and Anderson, 2002). We ranked candidate models according to ΔAICc (difference between model's AICc and min AICc). Lower ΔAICc indicates higher support for a given model. We evaluated the explanatory power of each model using Akaike weights (*wi*), which represent the probability that a candidate model is the best, given the data and the set of candidate models. We considered all models within 2 AICc of the best model(Burnham and Anderson, 2002).

We conducted all tests in R 2.14 (R Core Team, 2012); we fitted LMMs with the lme4 package (Bates et. al., 2011).

2.11. Demography model

We developed a linear, deterministic stage-structured matrix population model: $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$, where $\mathbf{n}_t$ is a vector that quantifies the number of individuals in each stage class at time t, and **A** is a transition matrix for each SAR (Caswell, 2001). We used annual matrices for *Agrimonia rostellata, Aristolochia serpentaria* and *Trillium erectum* and a seasonal matrix for *Carex retroflexa*

All populations experience periodic environmental variation, as seasonality within the year (i.e., *intra*-annual variation), or as a component of *inter*-annual variation. Such populations can be modeled using periodic matrix products (Caswell, 2001; Smith et. al., 2005). In studies of within-year seasonal variation (typically important in most plant species), the vital rates are measured over intervals shorter than a year and combined to describe growth over an annual cycle. In studies of inter-annual variability, the matrices are typically available for several years, or under several environmental conditions. Assuming that they reflect the range of environments encountered, in either case the matrices are multiplied together to describe population growth in an environment that cycles among the observed environmental states.

We refer to the annual cycle as consisting of *m* successive phases (e.g., a one year cycle could be divided into *m*=12 months). Each phase *i* is described by a matrix $\mathbf{B}_i$ that projects the population from phase *i* to phase *i*+1, and the last matrix $\mathbf{B}_m$ projects from phase *m* back to phase 1. The dynamics over an entire cycle starting at a given phase are described by multiplying the $\mathbf{B}_i$ around the cycle, in order, to form a periodic matrix product. The matrix product $\mathbf{A}_1 = \mathbf{B}_4 \ldots \mathbf{B}_1$ projects the population through the entire cycle. The subscript on $\mathbf{A}_i$ indicates that the projection starts at phase *i*. The advantage of a seasonal (periodic) modeling

approach is that it allows us to partition sources of variation in population growth among events occurring at different times of the year; this is not possible using standard annual projection models. Parameterizing seasonal matrix models (the $\mathbf{B}_i$ referred to above) requires collecting demographic data at various times of the year. Therefore, we collected data for *C. retroflexa* in November and June of each study year.

2.11.1. Parametizing the model

To parameterize the model we used adult survival and size data collected for each SAR population (section 2.8), size and juvenile survival for transplanted individuals (section 2.7) and germination rates from the seeding experiment (section 2.6).

To test our hypotheses concerning the relationship of treatment (fenced, open) on probability of transition among developmental states, we constructed a priori a set of 16 candidate approximating models, which we fit to the data. Our model set consisted of a general model with full time-interaction between transition parameter, year and fencing, and a series of nested, reduced parameter models which were symmetrical for all main effects and interactions of main effects. All approximating models were ranked using the Akaike Information Criterion, corrected for sample size (AICc). Models with AIC values which differed by < 2 were deemed to be equivalent. We assessed the relative importance of individual predictor variables in our models by summing the normalized AIC weights of among our candidate models; importance of a variable can be refined by making inference from all the models in the candidate set Akaike weights are summed for all models containing predictor variable (i.e., factor) x_j, j = 1,…, R. We denote these sums as w_+(j). The predictor variable with the largest predictor weight, w_+(j), is estimated to be the most important, while the variable with the smallest sum is estimated to be the least important predictor. Proportional support can be assessed by scaling individual variable sums relative to either largest or lowest sum among variables in the subset of models being considered. This approach can be extended to subsets of variables, including contributions to interaction terms, and is generally believed robust conditional that the candidate model set is symmetrical, or nearly so, with respect to model terms ((Burnham and Anderson, 2002)).

Transitions were based on numerical optimization of a multinomial likelihood where transition probabilities were first-order Markov. We used a multinomial logit link function to constrain transition estimates for a given state to sum to 1.0 during the numerical optimization of the likelihood. To minimize model selection uncertainty, we used model averaged estimates based on normalized AIC weights.

2.11.2. Assessing impact on plant demography

We applied standard perturbation techniques (sensitivity and elasticity analysis) to determine those factors that would have the greatest impact on population growth (λ). Applied to an annual or seasonal matrix model, we determined factors in a given season to which population growth is most sensitive.

While perturbation analysis provides a robust indication of the sensitivity of population growth to perturbation, sensitivity or elasticity analyses are prospective analyses, since they quantify the *expected* degree of perturbation to population growth given a specified change in one (or more) element(s) of the matrix. However, the vital rate which contributes most to the

observed variability in life histories is not necessarily the one to which life histories are the most sensitive, nor the one that will necessarily make the biggest contribution to variability in another environment (Horvitz et. al., 1996; Caswell, 2000).

One approach for partitioning variation in growth rates is the life table response experiment (LTRE), (Horvitz et. al., 1996; Caswell, 2000; Cooch et. al., 2001). The LTRE relies on the fact that if projected population growth rate is measured as a deviation from a reference value, then ‘‘treatment’’ effects (i.e., variation in projected growth rate) can be decomposed into contributions from each of the vital rates, in a manner structurally (but not formally) analogous to analysis of variance. To summarize how λ varied among fenced and open populations and among populations growing at sites with low and high earthworm density we used a two-way, fixed-design LTRE. The model is:

$$\lambda^{(m)} = \lambda^{(..)} + \lambda^{(m)}\ \lambda^{(n)} + \lambda^{(mn)}$$

where $\lambda^{(..)}$ is the growth rate of the overall mean matrix, $\lambda^{(m)}$ and $\lambda^{(n)}$ are the main effect of fencing treatment *m* and earthworm density *n*, measured as a deviation from the growth rate of the reference matrix, and $\lambda^{(mn)}$ is the residual interaction term. The effect $\lambda^{(m)}$ measures the effect of treatment *m* on λ, and incorporates **all** the differences in survival and fertility between the treatment matrix and the reference matrix. To decompose $\lambda^{(m)}$ into the contributions due to the differences in each matrix element, it can be shown that to first-order

$$\begin{aligned}\alpha^{(m)} &= \lambda^{(m)} - \lambda^{(.)} \\ &= \sum_{i,j}\left(a_{ij}^{(m)} - a_{ij}^{(.)}\right)\left.\frac{\partial\lambda}{\partial a_{ij}}\right|_{(1/2)\left(\mathbf{A}^{m}+\mathbf{A}^{.}\right)}\end{aligned}$$

The difference term in the summation represents the relative contribution of each element of the matrix (representing effects of mortality and survival for a given age or size class) to the deviation of the projected growth of the treatment matrix from the reference matrix.

3. Results and Discussion

3.1. Soil analyses

Soil composition between paired open and fenced plots was similar at the start of the experiment (P>0.05 for all measured components, **Fig. 10**). Sites dominated by non-native vegetation (N=6, see section 3.3.1 Vegetation monitoring) had significantly lower concentrations of Al ($F_{1,17}$ = 17.59, P=6-04) and Fe ($F_{1,17}$ = 19.1, P=4-04) and significantly higher Ca concentration ($F_{1,17}$ = 6.04, P=0.02) and pH ($F_{1,17}$ = 47.42, P=2-06) than sites dominated by native vegetation (**Fig. 10**). Sites with high earthworm density (N=8, see section 3.3.3) for full description of earthworm communities) had higher Al ($F_{1,17}$= 8.85, P=0.008) and Mn ($F_{1,17}$= 5.61, P=0.03) than sites with low earthworm density (N=4, **Fig. 10**). We found no significant interactions between studied factors. Nitrates (NO_3) were undetectable at six study sites and therefore we did not formally test for NO_3 effects.

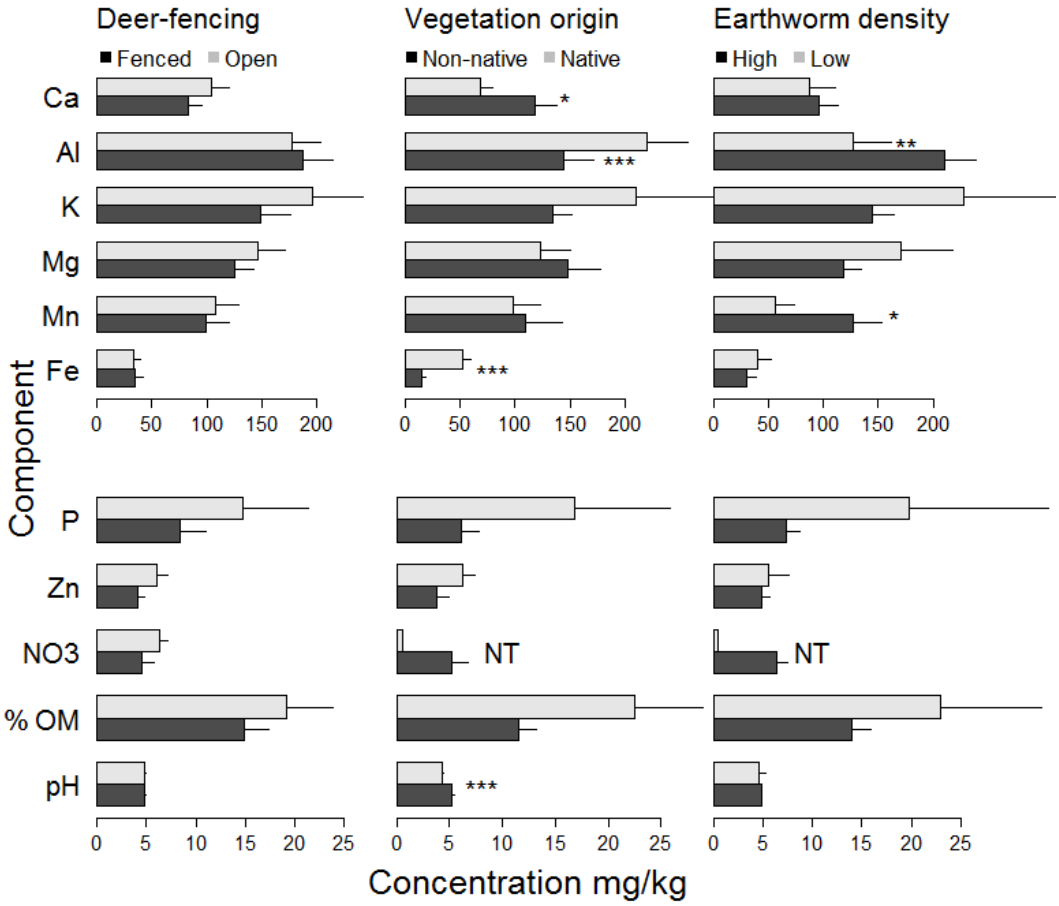

Fig. 10. Soil analyses results from samples collected at paired open and fenced plots at 12 sites at West Point NY on 28-31 October 2008. Sites varied according to dominant vegetation origin (N=6 sites per level) and earthworm density (N high=8, N low=4). Components were measured in mg/kg, except for pH and for OM (organic matter loss on ignition) which is presented as percent. * P<0.05, ** P<0.01, *** P<0.001, NT not tested. Note different scales between the top and bottom panels. Data are means + 2SE. For graphical purposes only, Ca concentration was divided by 10.

3.2. Seed bank study

A total of 2115 seedlings germinated over the course of the study, consisting of 86 species or morphospecies (1770 germinants), unidentified *Carex* (207 germinants) and seedlings identified only to life form (61 forbs and 74 graminoids; 6% of total germinants: excluded from total species count). The majority (91.4%) germinated in 2009, and the remainder in 2010 (6.4%) and 2011 (2.2%), demonstrating the relatively short survival of species in the seed bank. The most diverse genus in the seed bank was *Carex* (13% of germinants) with 13 identified species. The most abundant species was the non-native annual grass *M. vimineum* (30% of germinants). The two most widespread species, emerging from all sites, were *Betula lenta* L./*B.alleghaniensis* Britton (these two species could not reliably be separated at the seedling stage: 5% of germinants) and *Lobelia inflata* L. (13%).

Our 12 field sites varied in number of germinants (36 to 689; mean 176.3 ± 53.88) and in species richness (9 to 38 species; mean 24.6 ± 2.28). None of our chosen threatened or endangered species germinated from the seed bank. Germinant abundance varied according to life form ($F_{3,69}$=22.13; P<0.001), with most germinants belonging to graminoids (62% of all germinants grouped into 26 species), followed by annuals and biennials (14% and 18 species), perennial forbs (12% and 31 species) and woody species (7% and 12 species), which were considerably more common than ferns (0.4% in 3 species; **Fig. 11**). The majority of germinants were of native origin (63% native; $F_{1,69}$=98.23, P<0.001), and native germinants were more abundant at non-invaded sites (site invasion x origin interaction $F_{1,69}$=5.13, P=0.02).

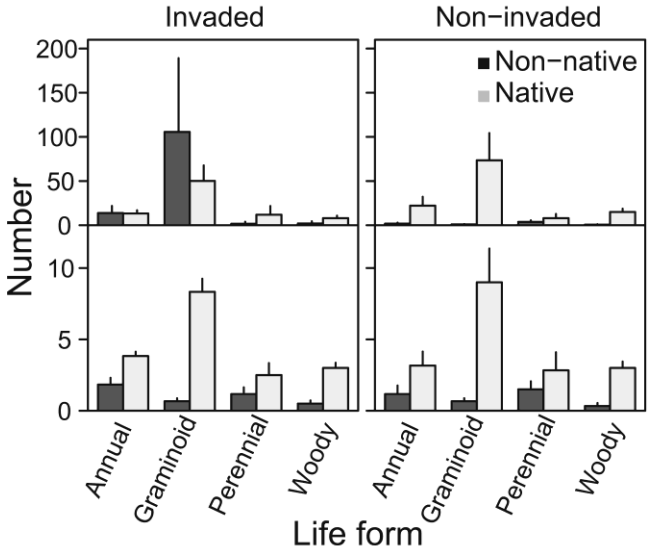

Fig. 11. Number (top) and species richness (bottom) of seed bank germinants according to life form, plant origin (non-native or native) and site invasion (invaded, non-invaded). Invaded sites (N=6, left panels) were dominated by non-native plant species: *A. petiolata* (N=2), *M. vimineum* (N=2) and *B. thunbergii* (N=2). Untransformed means + SE (N=6 sites/invasion level).

The best model for germinant abundance (SEM analysis) had an adequate fit of data to model (χ^2=8.68, P=0.97). Earthworm density had a positive effect on germinant numbers of all life forms of native and non-native origin, except for non-native graminoids (β or un-

standardized coefficient= 0.48 ± 0.11, P<0.001) (**Fig. 12a**). Total plant cover had a positive direct effect only on non-native graminoid germinant abundance (β= 2.79 ± 0.42, P<0.001), while species richness had a positive effect on the latter group and on native non-graminoids (β= 0.33 ± 0.06, P<0.001). As expected, earthworm density was positively correlated with non-native plant cover (β= 0.36 ± 0.15, P<0.05). Total plant cover significantly increased with introduced cover (β= 0.80 ± 0.02, P<0.001), while total species richness was positively related to total cover (β= 7.46 ± 2.41, P<0.01), but negatively affected by introduced cover (β= -6.73 ± 2.16, P<0.01; **Fig. 12b**).

Selected model for species richness had a good fit from data to the model (χ^2=14.91, P=0.78). Models explaining germinant species richness for native graminoid and non-graminoid species did not differ (P>0.05), hence both groups were collapsed into one group, hereafter referred to as natives. Species richness for native germinants was explained by a direct positive effect of earthworm density (β= 0.26 ± 0.05, P<0.001) and species richness (β= 0.08 ± 0.02, P<0.0; **Fig. 12**). Earthworm density significantly increased species richness of non-native non-graminoid germinants (β= 0.26 ± 0.05, P<0.01), but no other factor had a direct effect on this group. We did not test for effects on species richness of non-native graminoid germinants, as we only recorded one species in this category (*M. vimineum*).

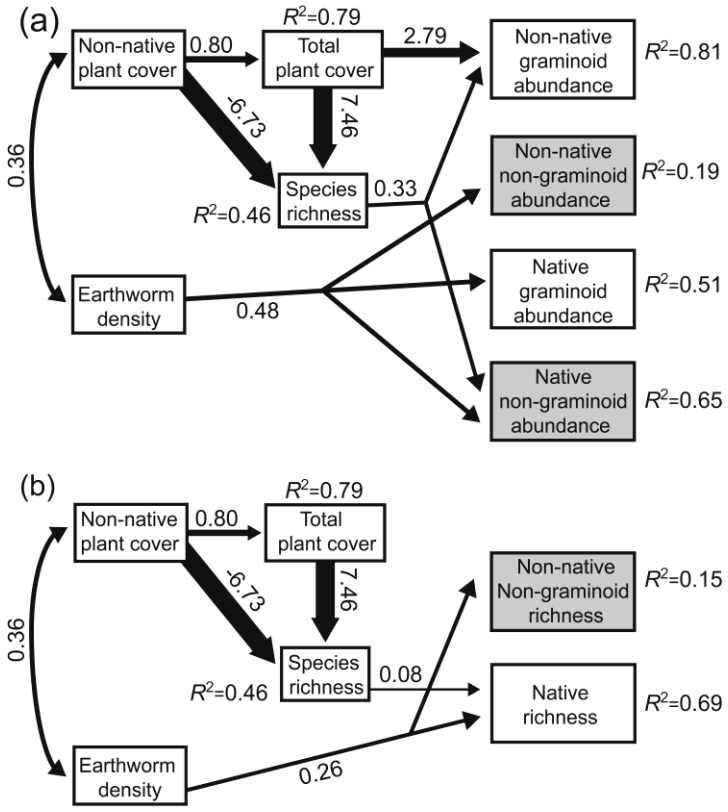

Fig. 12. Structural equation model results for (a) abundance and (b) species richness of germinants identified from soil cores collected at 12 sites at West Point NY in October 2008. Straight arrows represent direct effects of one variable on another, and curved arrows represent correlations. We only show significant pathways. To allow comparisons between groups we show unstandardized parameters. Thickness of arrows is proportional to the magnitude of coefficients. Native group for species richness model include graminoid and non-graminoid species. Non-graminoid species are depicted in light grey.

The abundance of native graminoid germinants was positively associated with increased earthworm abundance but only in the absence of invasive plants. Graminoid species richness in the seed bank is correlated with forest species richness. Thus, the greater the diversity within a forest the higher the predicted diversity of sedges and other graminoids in the seed bank.

Density and species richness of both native and non-native plant species germinating from the seed bank were positively associated with increased earthworm density. This relationship held for all life form groups, and was strongest for graminoids. Non-native plants were positively correlated with earthworm density, but directly influenced only the number of non-native germinants.

PERMANOVA results indicated significant differences in germinant composition between sites with low and high earthworm density ($F_{1,11}$=2.16, R^2=0.16, P=0.03), but no differences in germinant composition between sites with different levels of plant invasion ($F_{1,11}$=1.48, R^2=0.12, P=0.12) or a significant interaction between earthworm and plant invasion ($F_{1,11}$=1.11, R^2=0.08, P=0.35). Of the 90 species identified in the seed bank, 34 were only present at sites with high earthworm density and 13 were only present at sites with low earthworm density. Results for aboveground vegetation were not significant for all factors (P>0.05 for all cases).

Native plant species that responded positively to high earthworm density included early successional herbs (*Lobelia inflata, Oxalis stricta* L.) and graminoids (*Juncus tenuis* Willd., *Carex. albicans* Willd. ex Spreng*., C. appalachica* J. Webber & P.W. Ball and *C. swanii* (Fernald) Mack). Only one native species, the early successional tree *Betula* sp., had markedly higher germination in soil with low earthworm density than in soils with high earthworm density.

Shannon and Simpson diversity index, as well as Pielou and Sheldon evenness indexes varied across study sites (1.5 to 3.9 for Shannon, 0.09 to 0.6 for Simpson, 0.33 to 0.9 for Pielou and 0.20 to 2.25 for Sheldon), but neither index was affected by plant invasion, earthworm density or their interaction (P>0.5 for all cases). However, Simpson and Pielou indexes differed between aboveground vegetation plots and seed bank: Simpson index was higher for the aboveground vegetation than the seed bank (0.44 vs. 0.23; $F_{1,22}$=5.21; P=0.03) and Pielou index was lower (0.45 vs. 0.71, $F_{1,22}$=11.58, P=0.003).

Correspondence between vegetation plots and seed bank germinants (measured through the Jaccard index) was low and it depended on site invasion ($F_{1,81}$=7.51, P=0.007) and life form ($F_{3,81}$=6.91, P<0.001), but we found no interaction between both factors. Graminoid species presented higher correspondence than perennial and woody species, but did not differ from annuals (**Fig. 13**). Overall, total species richness was lower in the seed bank than in the vegetation plots (84 vs. 123 excluding morphospecies, respectively). In total, we identified 171 plant species in the seed bank and vegetation plots, of which 87 were present only in the vegetation plots, 48 were present only in the seed bank, and 36 were common to the seed bank and vegetation plots. Two introduced species: *M. vimineum* and *B. thunbergii* had the highest cover of aboveground vegetation; the first one was also the most abundant germinant species. Notably, we did not register *B. thunbergii* germinants, even though this species was fairly abundant at two study sites, attaining 21 and 95% cover.

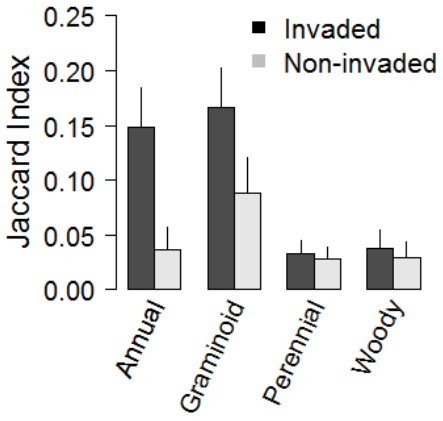

Fig. 13. Jaccard index (correspondence between aboveground vegetation and seed bank germinants) according site invasion and life form. Data are mean + SE (N=6 sites/invasion level). Note that the non-native *A. petiolata* was the most abundant annual/biennial germinant, and the non-native *M. vimineum* was the only graminoid germinant.

Our results suggest that forest seed bank composition is strongly influenced by the presence and abundance of non-native earthworms. Earthworm invasion may contribute to recovery of many species by facilitating germination, albeit for only a subset of the (original) species composition of the standing vegetation. A greater threat to forest recovery from the seed bank is the absence or extremely low abundance of many species, especially late-successional herbs and species with double-dormancy. In many Northeastern forests, including all 12 forests in this study, intense deer herbivory prevents flowering and fruit production of these herbs, and thus likely contributes to the rarity of the species in the seed bank. Our germination studies show that germination rates of these species, possibly with the exception of *C. retroflexa*, are more than adequate and seed viability does not appear to play a major role in the rarity of the species at our sites.

Our findings contrast with numerous studies that earthworms decrease seed germination and seedling survival (Milcu et. al., 2006; Regnier et. al., 2008; Eisenhauer et. al., 2012), albeit these effects are strongly earthworm species-specific (Eisenhauer et. al., 2009a; Forey et. al., 2011). The conflicting results may be due to the experimental design: we collected soil cores from sites with differing earthworm densities, and subsequently germinated the soil cores in the absence of earthworms. This approach allowed us to disentangle earthworm impact on seed bank composition from impact on seed germination and seedling survival. The majority of studies assessing earthworm impact on seed banks address this latter concern, by exposing seeds to earthworms and monitoring germination and seedling survival rates (Eisenhauer et. al., 2009b; Asshoff et. al., 2010; Eisenhauer et. al., 2012; Mudrak et. al., 2012). Under these conditions, seed germination rates are typically lower in the presence of earthworms (Milcu et. al., 2006; Eisenhauer et. al., 2012).

To our knowledge, only two other studies have investigated impact of earthworm presence in forest soil on subsequent germination. Eisenhauer et al. (2009b) found that grass and herb seedlings had significantly higher emergence rates from soils previously occupied by the earthworm *Octolasion tyrtaeum* Savigny, and that grasses failed to germinate from soils occupied solely by *L. terrestris*. Hopfensberger et al. (2011) found that germination rates from soil occupied by *Lumbricus* spp. were much lower than from soils where these worms were

absent. In both studies, overall germination rates were too low to permit assessment of individual plant species response to earthworm presence, and the focus was on earthworm species impact on germinant density. In our study, high germination rates allowed us to assess response of individual plant species to earthworm density, but not to earthworm identity. Furthermore, the choice of experimental plant species, and hence the effect of earthworms on consumption and germination, will greatly affect the outcome.

3.3. Field monitoring

3.3.1. Vegetation monitoring

Over the five year study period we found 187 species across all sites. Species richness per quadrat ($1m^2$) varied between sites, but did not differ between years, open and fenced plots or among sites with different earthworm densities (**Table 8, Fig. 14**). Likewise, diversity and evenness indexes (Shannon, Simpson and Pielou) also varied among sites and years, but were not affected by fencing or earthworm density and did not differ between sampling months (May or July) ($P>0.05$ for all cases).

Table 8. Model results for the effects of fencing and earthworm density on vegetation species richness, height (cm) and cover (%) and on leaf litter volume (cm^3). We fitted GLMM models with Normal errors for all response variables, except for species richness (Poisson errors). All models included site and plot within site as random factors.

Response	Factor	May			July		
		Estimate	SE	Statistic[a]	Estimate	SE	Statistic
Species richness	Intercept	1.65	0.15	11.09	1.58	0.16	9.96
Vegetation height	Intercept	30.05	7.54	3.99	37.72	7.47	4.38
	Year	4.35	0.86	5.09	4.27	0.85	5.04
Vegetation cover (%)	Intercept	30.46	6.41	4.76	37.14	7.60	4.89
	Year	7.22	0.92	7.78	3.57	1.22	2.92
Native vegatation cover (%)	Intercept	22.89	4.36	5.26	27.51	5.22	5.27
	Year	7.54	0.88	8.60	8.01	1.16	6.91
	Fencing	-5.96	3.10	-1.95	-7.66	3.26	-2.35
Leaf litter volume	Intercept	28.29	1.02	27.87	21.07	2.24	9.41
	Year L[b]	-2.25	0.79	-2.86	-1.53	0.85	-1.8
	Year Q	5.08	0.80	6.37	3.44	0.84	4.08
	Year C	2.99	0.81	3.71	2.16	0.8	2.7
	Fencing				-1.68	1.34	-1.25
	Earthworm				3.46	3.86	0.9
	F x E				5.3	2.18	2.43

[a]t and z values for GLMM with Normal and Poisson, respectively
[b]Polynomial effect of year: L linear, Q quadratic, C cubic

Mean vegetation height and leaf litter volume in May varied only by year, but leaf litter in July was significantly higher in the open plots of sites with low earthworm density than the remaining plots (significant interaction between fencing and earthworm density (**Table 8, Fig. 15**). While total vegetation cover (%) only varied by year and did not differ between open and fenced plots, native plant cover significantly increased in the fenced plots both in May and July (**Table 8**, **Fig. 14**).

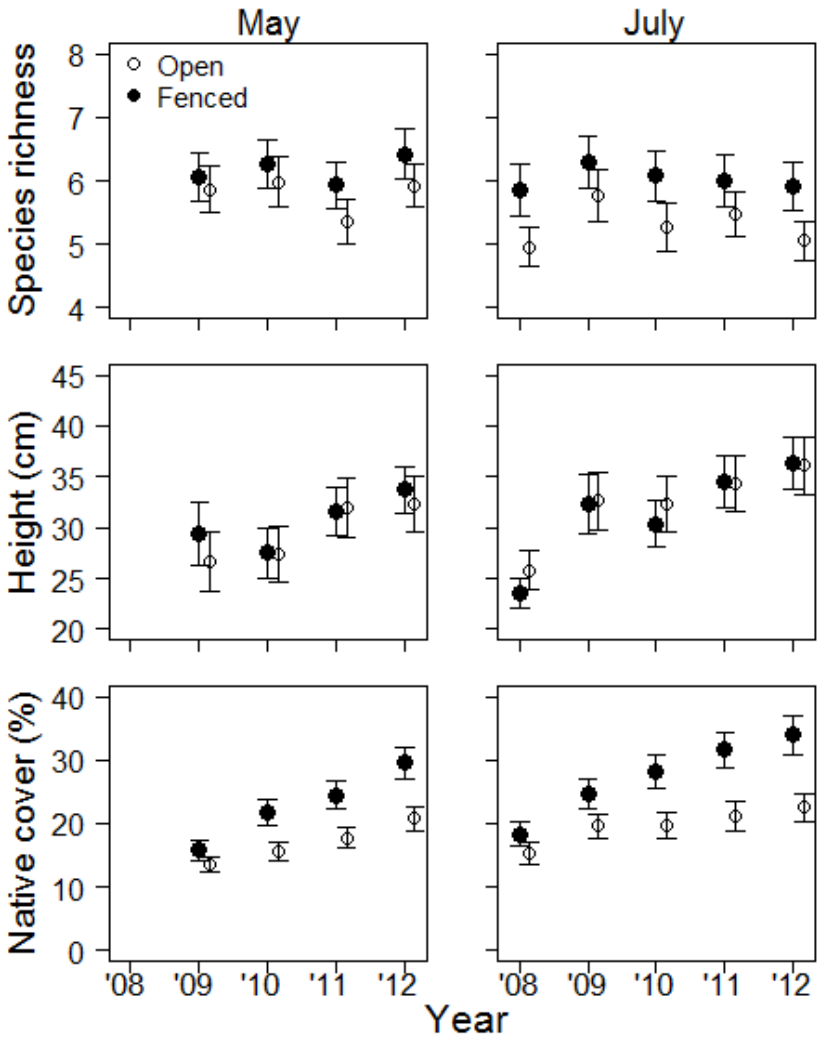

Fig. 14. Species richness, vegetation height (cm) and native vegetation cover (%) in open and fenced plots (n=10 quadrats/plot) in May (left panels) and July (right panels) at 12 sites at West Point, NY from 2008-2012. May surveys were conducted from 2009-2012. Due to plot relocation we excluded two sites (6 and 8) in July 2008. Data are means ± 1 SE. Points are jittered to allow visualization. Note different scales on each panel.

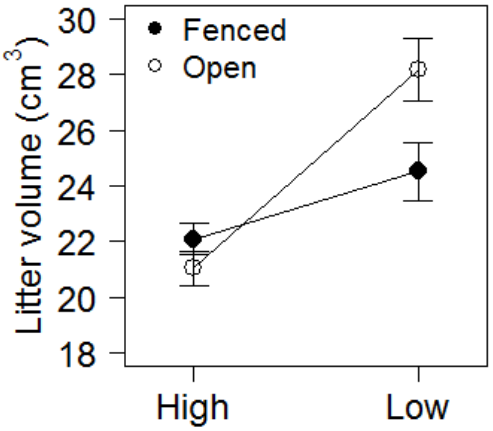

Fig. 15. Leaf litter volume (cm^3) in open and fenced plots in July at 12 sites with low and high earthworm density (N high=8, N low=4) at West Point, NY from 2008-2012. Data are means ± 1 SE of 10 quadrats/plot x site.

Non-native plant responses to fencing depended on species identity. We observed a significant reduction in *M. vimineum* cover (%), and an associated increase in cover of native ground-layer vegetation, inside fenced plots. This effect became more pronounced over time but was not affected by the sampling season (May or July, **Table 9**, **Fig. 16**). Total cover (introduced plus native vegetation combined) varied among sites but showed little annual variation and hence cannot explain the observed pattern. Consistent with proportional cover results (**Fig. 16**), we also found higher *M. vimineum* biomass at open plots (see section 3.3.2 on plant vigor). Our data also suggest a potential response by the perennial *B. thunbergii*: Five years after fencing there is a clear trend, although not significant, indicating reduced cover of the non-native *B. thunbergii* in fenced plots but stable cover in open plots. This trend is also confirmed by ring-growth analyses, which indicate higher radial growth at open plots (see section on plant vigor 3.3.2). We found an overall substantial reduction in *A. petiolata* cover at *A. petiolata* sites (**Fig. 16**) with no discernible fence effect. Such overall declines are now common at many invaded areas in the East and Midwest and appear a result of negative soil feedback (Blossey and Nuzzo, unpublished data).

Table 9. Model results for the effects of sampling year and fencing on *M. vimineum* cover: total vegetation cover. To meet model assumptions we square root-arcsine transformed *M. vimineum* proportional cover. Site and plot within site were included as random factors in GLMM model (Normal errors).

Predictor	Estimate	SE	T
Site	59.1	8.78	6.73
Year	-0.03	0	-6.71
Fencing	-48.43	12.51	-3.87
Year/month	0	0	-3.41
Year x Fencing	0.02	0.01	3.88
Year/month x Fencing	0	0	0.71

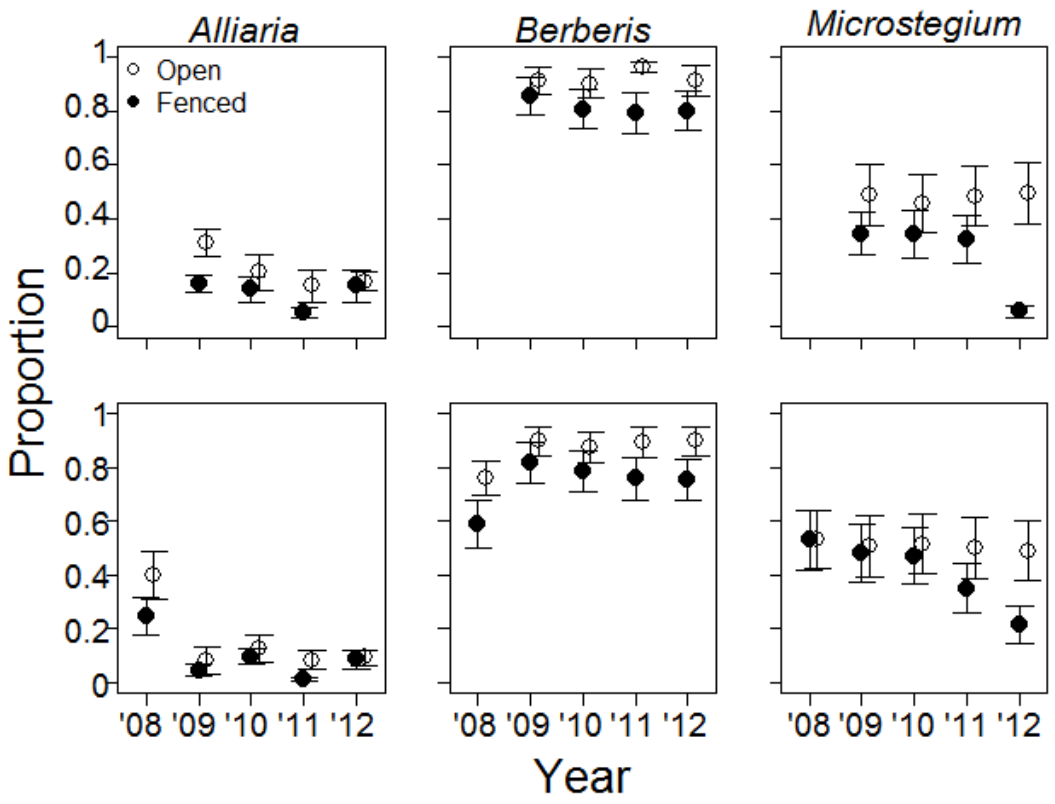

Fig. 16. Proportional contribution of *A. petiolata* (left), *B. thunbergii* (center) and *M. vimineum* (right) to total vegetative cover in May (top panels) and July (bottom panels) from 2008-2012 in open and fenced plots (N=2 sites/plant species with 10 permanent monitoring quadrats/plot) at West Point, NY. Data are means ± 1 SE.

The species composition of the understory layer in May and July differed among survey years (PerMANOVA, P<0.001 for both sampling months) and sites. PerMANOVA results indicated that species composition in May differed according to earthworm density (P<0.001) and fencing (P=0.01), with a marginally significant interaction between fencing and survey year (P=0.06) and between fencing and earthworm density (P=0.09). All other interactions were not significant.

In May, the species with highest cover at each site were similar at both the start (2009) and end (2012) of the study and between open and fenced plots, except at three sites with high native plant cover and high earthworm density (sites 8, 9 and 10). At site 8, *Carex pensylvanica* attained the highest cover in the fenced plot in 2009 and 2012 (3% and 4%, respectively), while in the open plot *Amphicarpaea bracteata* L. Fernald attained the highest cover in 2009 (4%) and *C. pensylvanica* in 2012 (6%). At site 9, *Eurybia divaricata* attained the highest cover in the open plot in both 2009 and 2012 (2% and 3%); while in the fenced plot *Erythronium americanum* (5%) and *Betula* sp. (9%) attained the highest cover in 2009 and 2012, respectively. At site 10, *E. americanum* Ker Gawl. was the species with highest cover in the fenced plot in 2009 (2%) and at both fenced and open plots in 2012 (2% and 1% respectively), and *Arisaema triphyllum* attained the highest cover in the open plot in 2009 (1%). *Vaccinium pallidum* Aiton was the most abundant species at two of the three sites with low earthworm density and primarily native vegetation cover (Site 2: 3%, Site 4: 10% across all plots

and years), while *Acer saccharum* attained the highest cover at the third site with these characteristics (Site 11: 13%). Not surprisingly, *B. thunbergii* (Site 1: 27%, Site 6: 82% across plots and years) and *M. vimineum* (Site 3: 10%; Site 12: 50%) attained the highest cover at the sites which were purposely selected because of the high cover of these non-native species. In contrast, *A. saccharum* (Site 5: 43% Site 7: 10%), not *A. petiolata*, attained the highest cover at sites selected because of initial high *A. petiolata* cover.

July surveys (2008 excluded from dataset) were similar to May, with some notable exceptions: At site 8, the species with most cover in both plots were *A. bracteata* in 2009 (8% and 10% for fenced and open plot, respectively) and *C. pensylvanica* in 2012 (8% and 10%, respectively). At site 9, *Betula* sp. had the highest cover in the fenced plots in both years (4% and 8%, respectively), whereas in the open plot the species with highest cover were *E. divaricata* in 2009 (1%) and *Fagus grandifolia* Ehrh. in 2012 (2%). At site 10, *Carex swanii* had the highest cover in the open plots in 2009 (0.40%) and 2012 (1%) while A. *triphyllum* (0.5%) and *F. grandifolia* (1%) attained the highest cover in the fenced plot in 2009 and 2012, respectively. At both sites dominated by *M. vimineum* the most abundant species in the fenced plots changed from *M. vimineum* (25% and 80% at sites 3 and 12, respectively) to *A. bracteata* (44%) at site 3 and to *Parthenocissus quinquefolia* (L.) Planch. (14%) at site 12. *M. vimineum* cover in the open plots was relatively constant throughout the study period (34 to 38% at site 3; 100% to 97% at site 12). PerMANOVA results indicated that species composition in July differed according to earthworm density ($P<0.001$) and fencing ($P<0.001$), with a significant interaction between fencing and earthworm density ($P<0.001$) and a marginally significant interaction between earthworm density and survey year ($P=0.06$). All other interactions were not significant.

Analyses of loser and winner species (G test) between the start (2009) and end of the study (2012) indicated changes in frequency of herb layer species between sites with different earthworm densities and between open and fenced plots. In May, at open plots, we found two losers: *E. americanum* and *C. swanii* and one winner: *P. quinquefolia* whereas at fenced plots we found one loser (*E. americanum*) and two winners (*Solidago caesia* L. and *P. quinquefolia*; **Fig. 17A**). In July, we found no winners or losers in the fenced plots, but two losers in the open plots: *Betula* sp. and *C. swanii* (**Fig. 18A**).

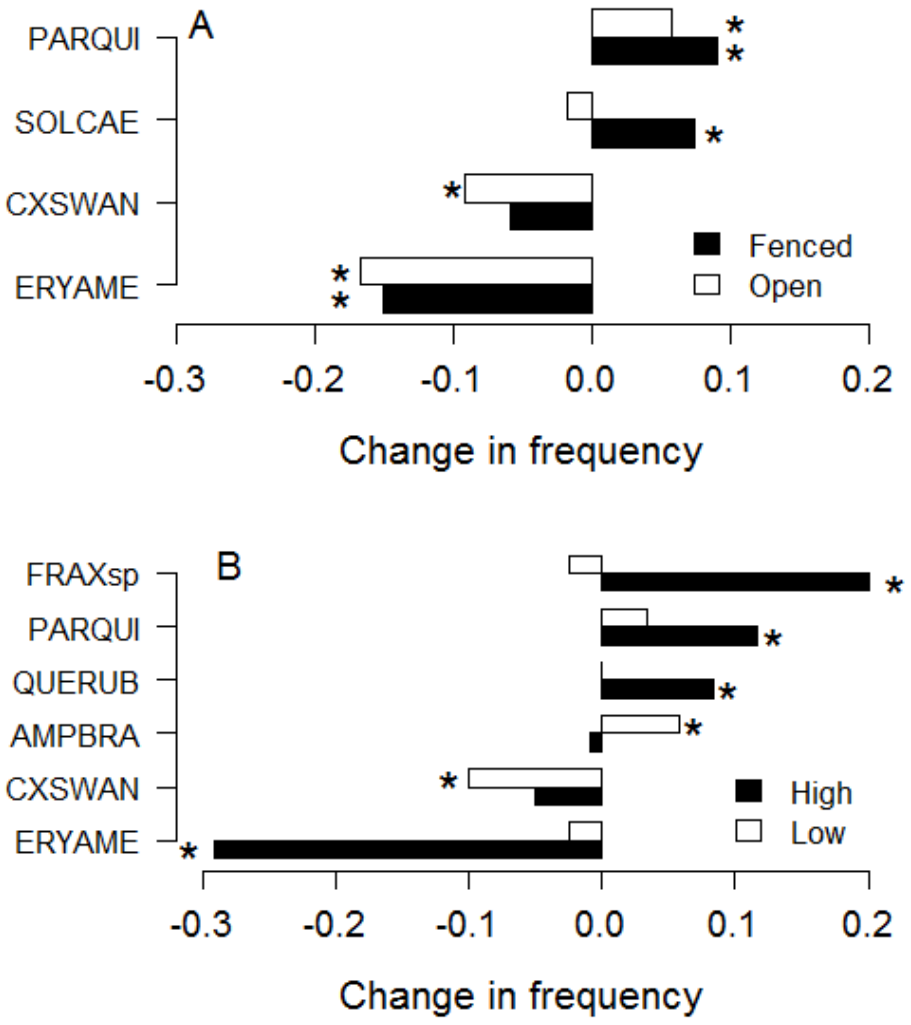

Fig. 17. Change in quadrat frequency in May of loser and winner species at (A) fenced and open plots at sites with (B) high (N=8) and low (N-4) earthworm density in West Point NY from 2009-2012 (N= 10 $1m^2$ quadrats/ open or fenced plot). * denotes significant G-test (adjusted P<0.05). Species ordered from highest to lowest change in frequency at the fenced plots and abbreviated as the three first letters of the genus followed by the first three letters of the species epithet.

In May, at sites with low earthworm density we found one winner (*A. bracteata*) and one loser (*C. swanii*) species, whereas at sites with high earthworm density we found one loser (*E. americanum*) and three winners (*Quercus rubra*, *P. quinquefolia and Fraxinus sp.)* (**Fig. 17A**). In July, we found three losers (*Betula sp., Acer rubrum* L. and *C. swanii)* and one winner (*P. quinquefolia)* at sites with low earthworm density. At sites with high earthworm density we found two losers (non-native *B. thunbergii* and native *Galium circaezans* Michx.) and two winners (non-native *A. petiolata* and native *Fraxinus sp.*) (**Fig. 18B**). Note that changes in frequency of the woody species *A. rubrum, B. thunbergii, Betula* sp., and *Fraxinus* sp., reflect changes in newly germinated seedlings and not established plants.

While understory vegetation differed in species diversity and composition across all sites, and we did see an increase in native vegetation cover during the five year study, we did

not see the expected increase in height or species diversity over time in response to fencing plots. This is contrary to our prediction and a finding that we are still unable to explain. There may be a multitude of factors that contribute to this apparent lack of response: including a phenotypic 'dwarfing' response to long-term deer herbivory of the most abundant plant species, and local rarity or extermination of species that are strongly negatively impacted by deer herbivory (particularly species in the Liliaceae family). We explored this relationship in 2012 by collecting detailed data for a subset of species that appear more vigorous inside the fenced plots than in the paired open plots (see section 2.4.2). Throughout the study period many plants did not flower (and thus failed to produce seed) in the fenced plots; and for the subset of species that flowered, flowering occurred primarily after several years of fencing. Thus, for the forests at West Point, five years of fencing is too short a time period for most plants (especially long-lived herbs) to successfully reproduce from seed. Most plants that increased in frequency in the fenced plots were tree seedlings that established from wind-dispersed seeds.

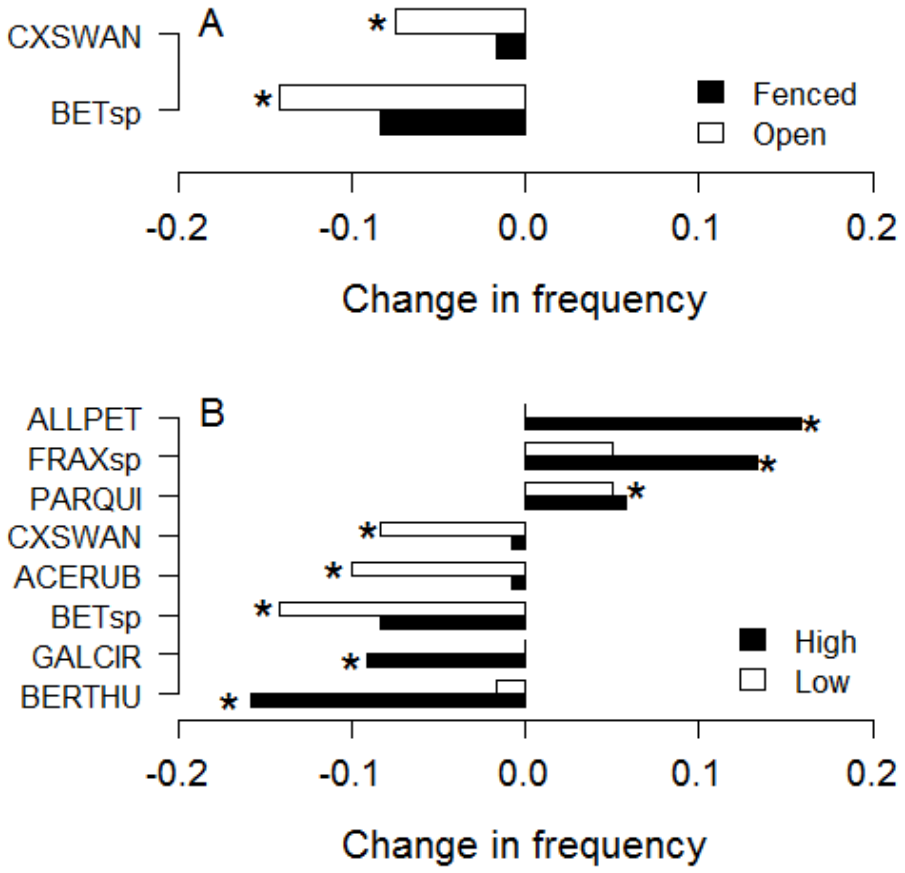

Fig. 18. Change in quadrat frequency in July of loser and winner species in (A) fenced and open plots at sites with (B) high (N=8) and low (N-4) earthworm density in West Point NY from 2009-2012 (N= 10 1m^2 quadrats/ open or fenced plot). * denotes significant G-test (adjusted P<0.05). Species ordered from highest to lowest change in frequency at the fenced plots and abbreviated as the three first letters of the genus followed by the first three letter of the species epithet.

3.3.2. Plant vigor

3.3.2.1. *Alliaria petiolata*

Alliaria petiolata cover varied in the 1.0m^2 permanent quadrats in all plots 2008-2012 reflecting the biennial nature of this plant and over time declined in 5 of the six plots (**Table 10**). Frequency also varied biennially, but in 2012 was similar to that recorded in 2008 (data not shown). However, based on the 20 3.14m^2 circular quadrats randomly established in 2012, *A. petiolata* frequency (G-test for Site 5 and 7 P<0.001, for Site 12 P=0.21) and density were significantly lower in the fenced plots relative to the open plots (**Table 11**, **Table 12**).

Table 10. *Alliaria petiolata* mean percent cover in in 10 1m^2 permanent quadrats in fenced and open plots at three sites at West Point in July, 2008 – 2012.

Site	Fencing	2008	2009	2010	2011	2012
5	Fenced	3.07	0.17	2.67	0.05	2.56
	Open	5.32	0.04	0.41	1.15	1.04
7	Fenced	3.22	0.49	1.66	0.15	0.96
	Open	8.19	0.99	3.14	1.06	2.74
12	Fenced	6.70	0.06	1.20	0.65	1.70
	Open	0.87	0.49	1.40	0.15	5.40

Table 11. *Alliaria petiolata* stem density/3.14 m^{-2} and frequency in 20 circular quadrats/plot at three sites in West Point in June 2012.

Site	Fencing	Density/3.14 m^2	Frequency
5	Fenced	0.15	0.10
	Open	2.60	0.55
7	Fenced	0.60	0.30
	Open	9.50	0.65
12	Fenced	2.15	0.35
	Open	1.20	0.55

In 2012, *A. petiolata* stem height averaged 49.95 ± 1.03 cm tall and reproductive plants produced on average 9.43 ± 0.45 siliques and 102.76 ± 5.67 seeds per plant. Plant height analyses identified one plausible model (AICc *wi*=1), which included site, fencing and their interaction (**Table 12**). While plant height was similar at two sites, it was significantly higher at the open plot of the third study site (Site 12, initially established to assess impact of *M. vimineum*, **Fig. 19**).

Table 12. Model results for the effects of site and fencing on garlic mustard (*A. petiolata*) density (GLM Negative binomial), height (LM) and silique number (GLM with Poisson errors). Density data is based on original 20 circular quadrats, height and silique number based on original 20 quadrats plus additional quadrats (see section 2.4.2 for collection methods).

Predictor	Density/3.14 m^2			Height			Silique number		
	Est.	SE	z	Est.	SE	t	Est.	SE	z
Site 5	-1.9	0.69	-2.76	60.72	4.14	14.67	-0.20	0.13	-1.59
Site 7	1.39	0.83	1.67	-18.26	5.80	-3.15	-0.33	0.10	-3.27
Site 12	2.66	0.8	3.35	-17.26	4.77	-3.62	-0.09	0.07	-1.30
Fencing	2.85	0.79	3.6	-5.82	4.73	-1.23	-0.16	0.06	-2.58
S7 x F (open)	-0.1	1	-0.1	9.12	6.40	1.43	0.39	0.11	3.63
S12 X F(open)	-3.44	0.99	-3.49	31.89	6.13	5.20	0.13	0.08	1.59
Height							0.05	0.00	15.24
Height square							0.00	0.00	-5.72

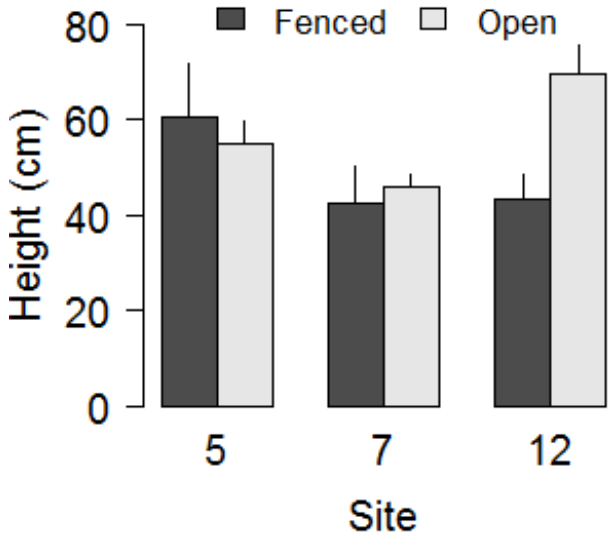

Fig. 19. *Alliaria petiolata* height (cm) as a function of site and deer-fencing in June 2012 at three sites at West Point NY.

The best model to explain plant silique number had 6.33 times the explanatory evidence of the next best model and it included plant height, and a site x fencing interaction (**Table 12**). At two sites there was no difference in number of siliques between open and fenced plots, but at the third site (Site 7) plants produced more siliques in the open plot (**Fig. 20**). The number of seeds was correlated with number of siliques per plant (Pearson coefficient 0.97) and followed a similar pattern (data not presented).

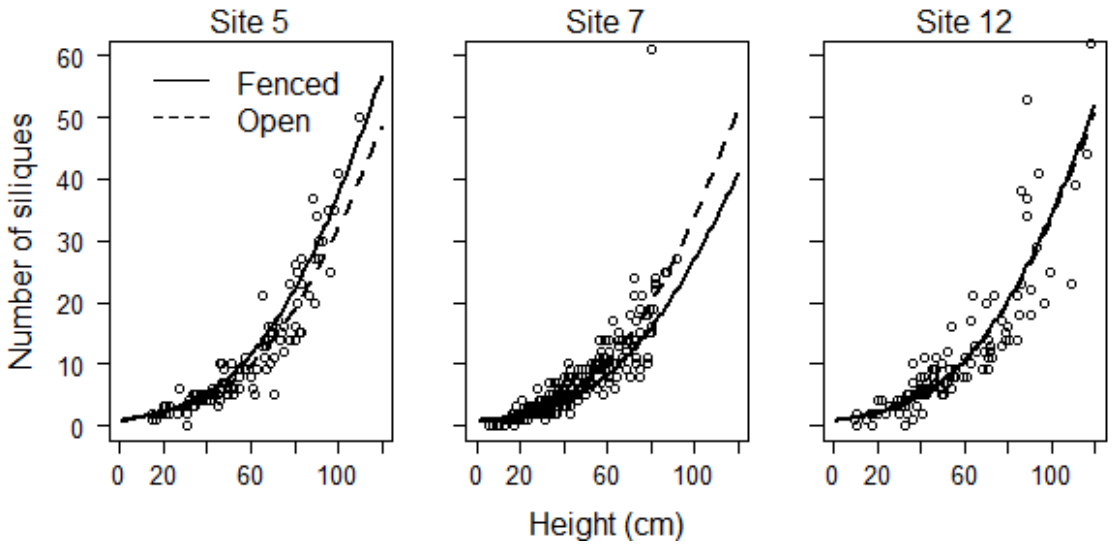

Fig. 20. Number of *A. petiolata* siliques according to plant height (cm) in open and deer-fenced plots at three sites at West Point in June 2012. Lines represent GLM with Poisson error predictions.

Thus, *A. petiolata* abundance declined significantly in the fenced plots compared to the open plots after five years of fencing. Note that this occurred in the two plots initially established to assess impact of *A. petiolata*; this decline did not occur in the plot initially established to assess impact of the non-native grass *M. vimineum*. Consequently, while individual plants had similar height and reproductive effort regardless of location, far fewer seeds were produced within the fenced plots, contributing to a downward cycle of reduced abundance. This was an unexpected consequence of fencing, and may be related to reduced earthworm density or to reduced deer trampling; additional experimentation is needed to determine the causative factors.

3.3.2.2. *Berberis thunbergii*

Bereberis thunbergii stem ages ranged from 1 to 18 years (1994-2011), with mean of 7.90 ± 3.98 years (**Table 2**). Only one model was included in the candidate set (AICc=1437.44, wi=0.69, ΔAICc to next model=2.44) and it included a significant effect of fencing and an interaction between fencing and earthworm density (**Table 13**). Age-standarized radial growth was higher in the open than in the fenced plots and at the open plots of sites with high earthworm density (**Fig. 21**). The difference between open and fenced plots occurred immediately after fence installation but did not increase thereafter: Age-standarized radial growth was 2.4 and 1.24 times higher in the open than in the fenced plots, in 2008 and in the 2009-2011 period, respectively (**Fig. 21**). Contrastingly, the interactive effect of fencing and earthworm density increased with time: By 2011 the open plots of high earthworm density sites had 2.5 times higher age-standarized radial growth than the remaining plots.

Table 13. Model results for the effects of fencing and earthworm density on *B. thunbergii* age-standardized radial growth.

Factor	Estimate	SE	t
Intercept	0.04	0.12	-0.33
Fencing	0.09	0.02	3.58
Earthworm	0.07	0.25	0.29
Fencing x Earthworm	-0.11	0.04	2.55

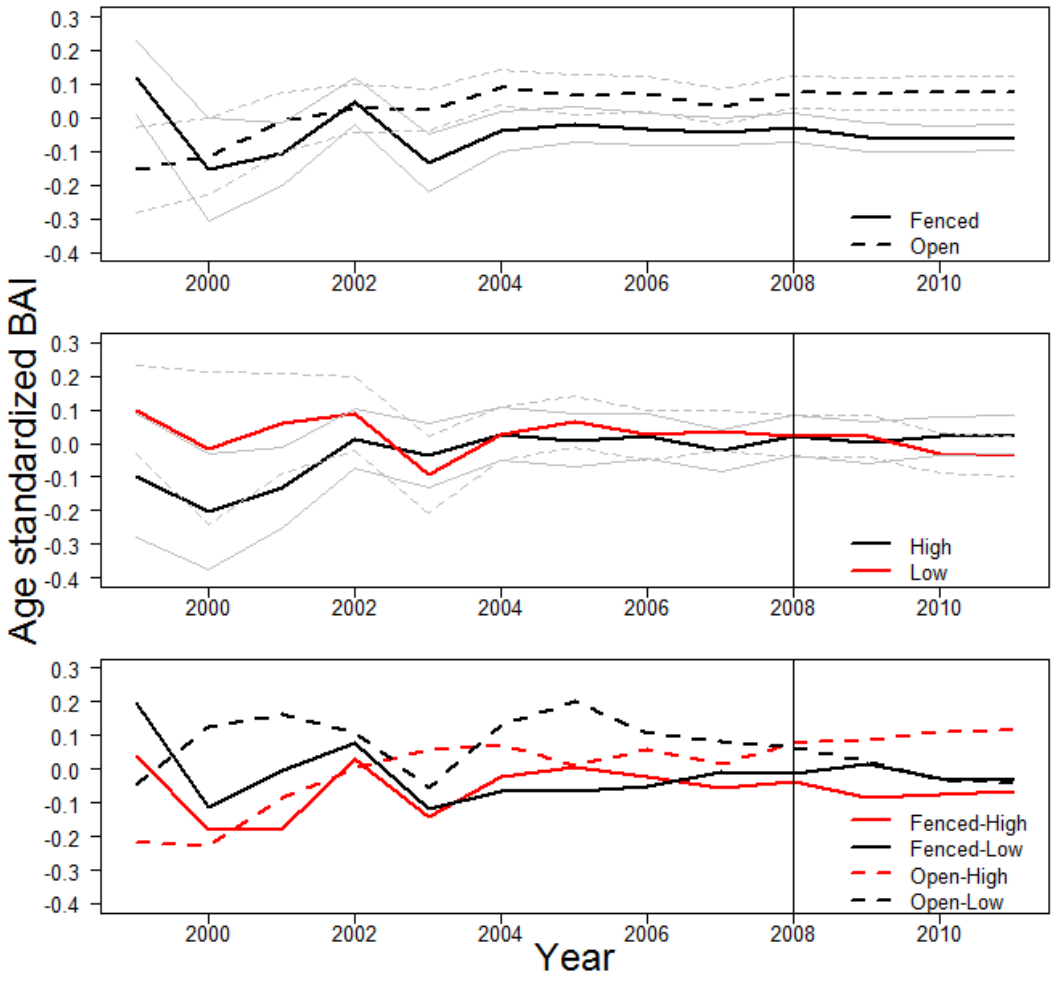

Fig. 21. Trend in age-standardized basal area increment (BAI ± SE) according to fencing (top), earthworm density (middle) and the interaction between both factors (bottom) of *B. thunbergii* stems collected at 8 sites at West Point, NY (N high earthworm density=6, N low earthworm=2). Data are shown for the period with at least 10 individuals in each treatment (1999–2011). The vertical line shows the start of experimental deer fencing. Black and red lines show the mean and grey lines the SE.

3.3.2.3. *Microstegium vimineum*

Japanese stilt grass (*M. vimineum*) mean cover declined over the five year period in the fenced plots, both absolutely and relative to the open plots, at the two sites initially established to assess impact of this non-native plant (Sites 3 and 12) (**Table 14**), and remained constant at the third site, initially established to assess impact of the non-native plant *B. thunbergii* (Site 1). Interestingly, frequency within the $1m^2$ quadrats at all 3 sites remained constant (data not shown); the reduced cover implies that the number and/or size of individual *M. vimineum* plants declined over time in the fenced plots. Biomass data collected in $0.25m^2$ quadrats indicates similar abundance in *M. vimineum* patches (reflecting the bias in quadrat location when plants were absent in the initial location; see section 2.4.2). Biomass (above and belowground mass combined) averaged 19.1 ± 3.8 g across all sites, but it was significantly higher in the open plot of one site (Site 12 open: 80.98 ± 5.35 g; site x fencing interaction: $F_{2,54}$=125.85, P<0.001).

Table 14. *Microstegium vimineum* mean percent cover in 10 $1m^2$ permanent quadrats in fenced and open plots at three sites at West Point in July, 2008 – 2012.

Site	Location	2008	2009	2010	2011	2012
1	Fenced	1.0	0.3	1.2	1.2	1.0
	Open	2.7	0.5	1.2	0.3	0.4
3	Fenced	29.0	25.2	24.6	10.6	16.4
	Open	45.2	33.9	46.1	30.1	38.3
12	Fenced	91.2	80.1	79.9	42.3	13.9
	Open	100	100	100	91.0	97.0

Thus, both *M. vimineum* and *A. petiolata* had reduced abundance in fenced plots dominated only by one non-native plant species. However, this trend was not observed in fenced plots dominated by two non-native plant species. At this point we cannot explain this pattern.

3.3.2.4. *Eurybia divaricata, Maianthemum racemosum* and *Polygonatum pubescens*

Between 2008 and 2012, *Eurybia divaricata* mean cover increased in all fenced plots, and remained relatively constant in all open plots (**Table 15**). Frequency remained constant in all plots during the same period (data not shown), implying that the increase in cover was due to growth of plants already present in the plots when fences were established in 2008. *Maianthemum racemosum* and *P. pubescens* were so uncommon in all plots that very few individuals were captured in the permanent quadrats (Site 5 and Site 8 only; data not shown). Plants of one or both species were observed within plots, but not quadrats, at Site 3 Fenced (one *M. racemosum*), Site 11 Fenced (three *M. racemosum* and two *P. pubescens*) and Site 11 Open (two *P. pubescens)*.

Table 15. *Eurybia divaricata* mean percent cover in 10 1m^2 permanent quadrats in fenced and open plots at 6 sites at West Point in July, 2008 – 2012 (Site 8 Open plot was established spring 2009).

Site	Fencing	2008	2009	2010	2011	2012
1	Fenced	0.2	1.0	1.3	1.6	1.5
	Open	0.6	1.0	0.9	0.6	0.3
3	Fenced	0.1	0.3	0.8	2.0	3.1
	Open	0.0	0.0	0.0	0.0	0.0
5	Fenced	0.2	0.7	1.3	1.4	1.1
	Open	0.5	0.1	0.1	1.0	1.0
8	Fenced	0.5	1.1	1.7	1.8	2.1
	Open		1.1	0.9	1.1	0.7
9	Fenced	1.4	2.0	2.0	2.2	3.4
	Open	1.7	1.3	1.5	1.1	1.7
11	Fenced	0.4	1.1	1.3	2.0	5.0
	Open	0.4	0.1	0.2	0.4	0.8

In total, we measured 942 *E. divaricata,* 50 *M. racemosum* and 109 *P. pubescens* stems. All three native herbaceous species responded favorably to protection from deer herbivory: Individual plants were taller and had wider leaves (not measured for *E. divaricata*) and were more likely to flower in the fenced than open plots at all sites (**Table 16, Fig. 22, Fig. 23**).

Table 16. Model results for the effects of site, fencing and their interaction on leaf width, plant height and probability of flowering of three native perennial plant species: *E.divaricata, M. racemosum* and *P. pubescens*.

	***Eurybia divaricata*[a]**		***Maianthemum racemosum*[b]**		***Polygonatum pubescens*[b]**	
	Estimate	**t**	**df**	**F**	**df**	**F**
Leaf width[d]						
Site			1,47	20.85***	1,105	56.35***
Fencing			1,47	11.12**	1,105	85.07***
Site x Fencing			NS[c]	NS	1,105	8.17**
Height						
Site	random	random	1,47	8.0**	1,106	35.21***
Fencing	-20.27 ± 3.54	-5.728	1,47	17.17***	1,106	46.23***
Site x Fencing	NS	NS	NS	NS	NS	NS

NS not significant, * P<0.05, ** P<0.01, *** P<0.001
[a]GLMM model with binomial errors; site and plot within site included as random factors.
[b]GLM model with binomial errors; site included as fixed factor.
[c]NS terms were dropped from the final model
[d]Leaf width was not measured for *E. divaricata*

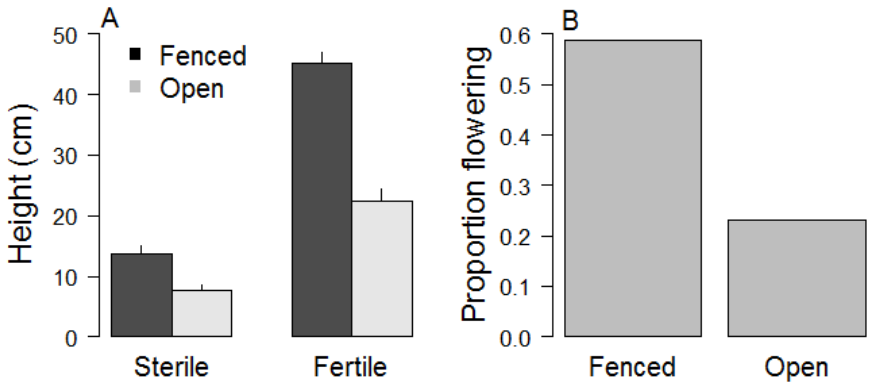

Fig. 22. Height of sterile and fertile *E. divaricata* individuals in open and fenced plots (A) and proportion of flowering plants in response to fencing (B) measured in open and fenced plots at six sites at West Point in June 2012. Data are means ± 2SE.

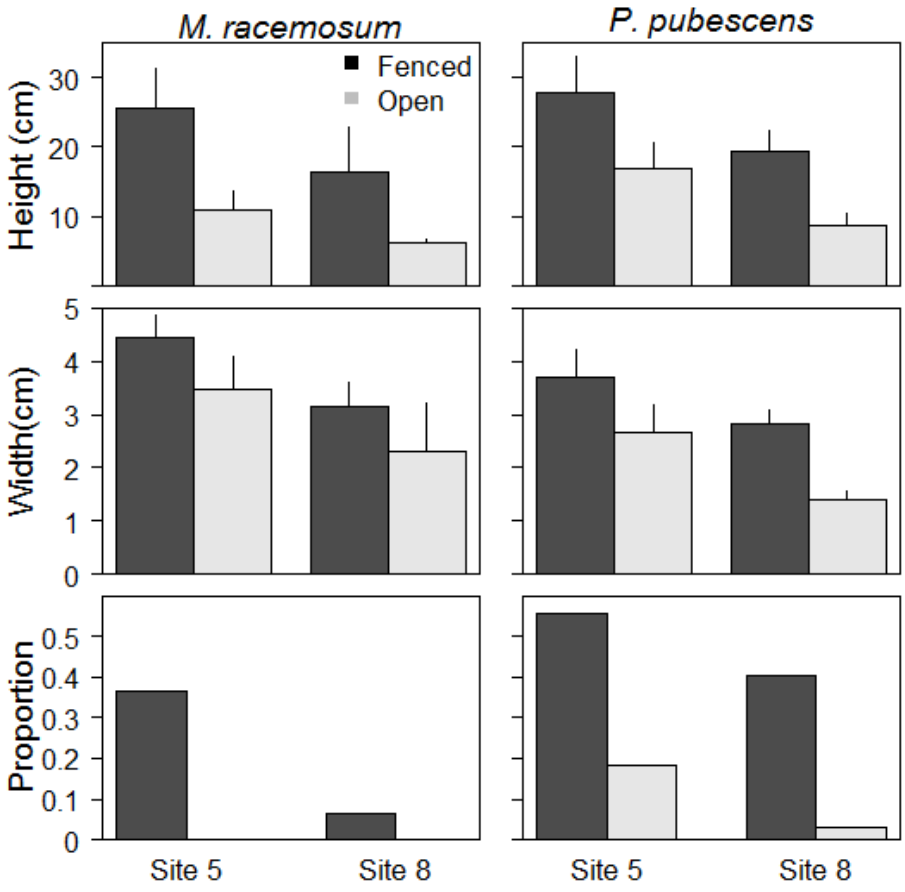

Fig. 23. Plant height (cm), leaf width (cm) and proportion of flowering plants of *M. racemosum* and *P. pubescens* in open and deer-fenced plots at two sites at West Point in June 2012. Data are mean ± 2SE.

The probabilities of flowering of the three plant species increased with plant height and varied according to site (**Table 17**). In general, a lower proportion of individuals flowered in the open (where plants were shorter) than in the fenced plots (**Fig. 23, Fig. 22**). *Eurybia divaricata* probability of flowering was higher in fenced plots and increased with plant height; interestingly, plants in the open plots were more likely to flower at a shorter height than plants of the same height in the fenced plots (**Fig. 22**). We did not find any flowering individuals of *M. racemosum* in the open plots of either site; therefore, we could not formally test for a deer-fencing effect. The best model for *M. racemosum* included an effect of plant height, site and their interaction and it had 1.61 more explanatory power than the next best model, which did not include the interaction term. Flowering probability of *P. pubescens* was best explained by height and site, this model had 2.66 times the explanatory evidence of the next best model which also included deer-fencing (**Table 17**).

Table 17. Model selection results for logistic regression analysis of flowering probability of *E. divaricata*, *M. racemosum* and *P. pubescens* as function of plant height, site and fencing.

Candidate models	K	AICc	ΔAICc	w_i
E. divaricata				
Height + fencing	5	486.62	0	0.84
Height * fencing	6	490.45	3.84	0.12
M. racemosum				
Height * site	4	29.09	0	0.50
Height + site	3	30.03	0.95	0.31
Height	2	30.98	1.89	0.19
P. pubescens				
Height + site	3	38.23	0	0.56
Height + site + fencing	4	40.23	2	0.21

After five years of fencing we detected significant changes in vegetation in response to fencing and to earthworm abundance. Native species increased in abundance (cover) density, and/or reproductive activity in the fenced plots, and all three target non-native plant species (*A. petiolata, B. thunbergii,* and *M. vimineum)* decreased in one or more measures of abundance (cover, density and/or frequency) in the fenced plots. At the same time, we found that individuals of native species responded positively to fencing by increasing in height and increasing their representative activity. While the plant community did not undergo drastic changes, the response of individuals of certain plant species clearly suggests the importance of deer herbivory for the reduced performance of the species that are suppressed, potentially for decades. The differences in the height needed to initiate flowering between plants in open and fenced plots further suggests that potential evolutionary interactions have occurred favoring plants with lower stature in areas where they are exposed to high deer herbivory. How these eco - evolutionary interactions play out over the long term, and how they could affect plant demography remains to be investigated. It is possible that decades of intensive deer browse have resulted in plants that are reasonably well adapted to deer browse but are poor performers or contributors to population growth rates due to reduced reproductive activity and stature. This may negatively affect the long-term viability of populations exposed to chronic deer herbivory.

3.3.3. Earthworm monitoring

Earthworm density (0.33 to 14.1 earthworms per 0.25 m^{-2} per site) and biomass (0.02 to 9.5 g per 0.25 m^{-2} per site) varied among sites and sampling years (**Fig. 24**). We recorded thirteen non-native earthworm species grouped into eight genera over the sampling period. The genera *Amynthas*, *Aporrectodea, Dendrobaena* and *Lumbricus* were the most abundant (**Fig. 25**). The epigeic *Amynthas* sp. (mean individual biomass 0.96 g) was present at eight sites, but was dominant at two of those sites accounting for 95 and 98% of collected earthworms (**Fig. 25**). The endogeic *Aporrectodea* (mean biomass 0.18 g) and the epigeic *Dendrobaena octaedra* Savigny (mean biomass 0.05 g) were dominant at three sites each, accounting for 45-85% and 41-49% of earthworm numbers, respectively. The anecic *L. terrestris* (mean adult biomass 2.75 g) was present at six sites in relatively lower abundance (1-17%). However, juveniles of this genus were common and accounted for 23% of all captured earthworms and for 15-27% of earthworms captured at each site (**Fig. 25**). *Lumbricus* spp. adults and juveniles were absent or at very low abundance at sites dominated by *Amynthas* sp.

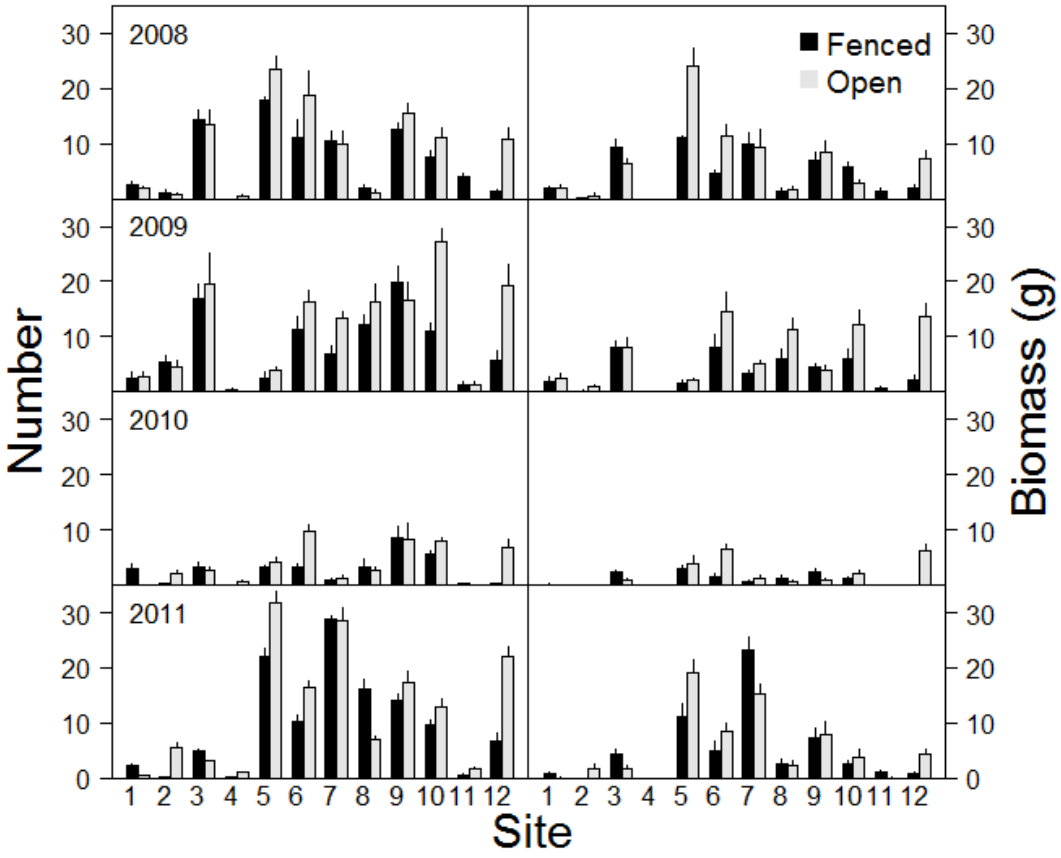

Fig. 24. Density (number per 0.25 m^{-2}) (left) and biomass (g per 0.25 m^{-2}) (right) of earthworms captured from 2008-2011 in fenced and open plots at each study site at West Point, NY. Data are untransformed means ± 1 SE (N=5 samples per site). Sites 1 and 6 are dominated by non-native *B. thunbergii,* sites 3 and 12 by *M. vimineum* and sites 5 and 7 by *A. petiolata*.

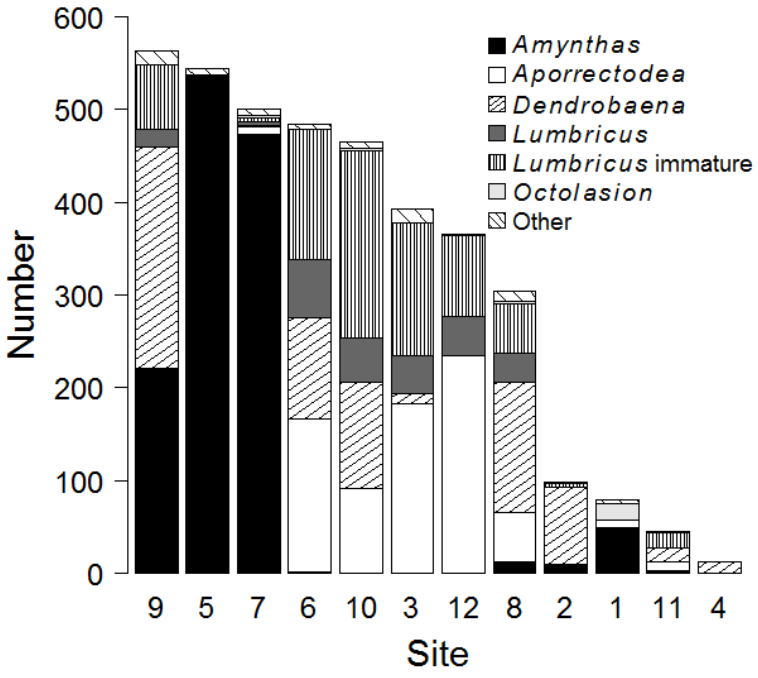

Fig. 25. Species composition of earthworm communities at 12 sites at West Point, NY from 2008-2011. Sites are ordered according to earthworm density. Sites 1 and 6 are dominated by non-native *B. thunbergii,* sites 3 and 12 by *M. vimineum,* and sites 5 and 7 by *A. petiolata*.

Earthworm density analyses indicated a significant effect of year, vegetation origin and fencing. Three models were included in the candidate set. Given the data, the best model included a polynomial effect of year, vegetation origin, fencing and an interaction between vegetation origin and sampling year and it had 1.77 times the explanatory power than the next model which also included a vegetation origin x fencing interaction (**Table 18**). Density was lower in 2010 compared to the other sampling years. Over the five year study period, earthworm density was on average 37% higher at sites dominated by non-native plants than at sites dominated by native vegetation and was 25% higher in open vs. fenced plots (**Table 19**, **Fig. 26**). The effect of non-native plants depended on sampling year: Earthworm density was higher at sites dominated by the non-native plants *A. petiolata, B. thunbergii and M. vimineum* only in 2008 and 2011 (**Fig. 26**).

Table 18. Model selection results for linear mixed model analyses of earthworm density and biomass at 12 sites at West Point, NY from 2008-2011. All models included site and plot (fenced or open) within site as random factors.

Response	Model	K	AICc	ΔAICc	w_i
Earthworm density	Y * VO + F	12	636.52	0	0.39
	Y * VO * F[a]	13	637.68	1.17	0.22
	Y * VO	11	637.90	1.39	0.20
Earthworm biomass	Y * VO + LLvol	12	189.87	0	0.46
	Y * VO + F + LLvol	13	190.56	0.69	0.33
	Y * VO * F[a] + LLvol	14	191.74	1.88	0.18

F fencing; LLvol leaf litter volume, VO vegetation origin; Y year
[a]second level interactions only

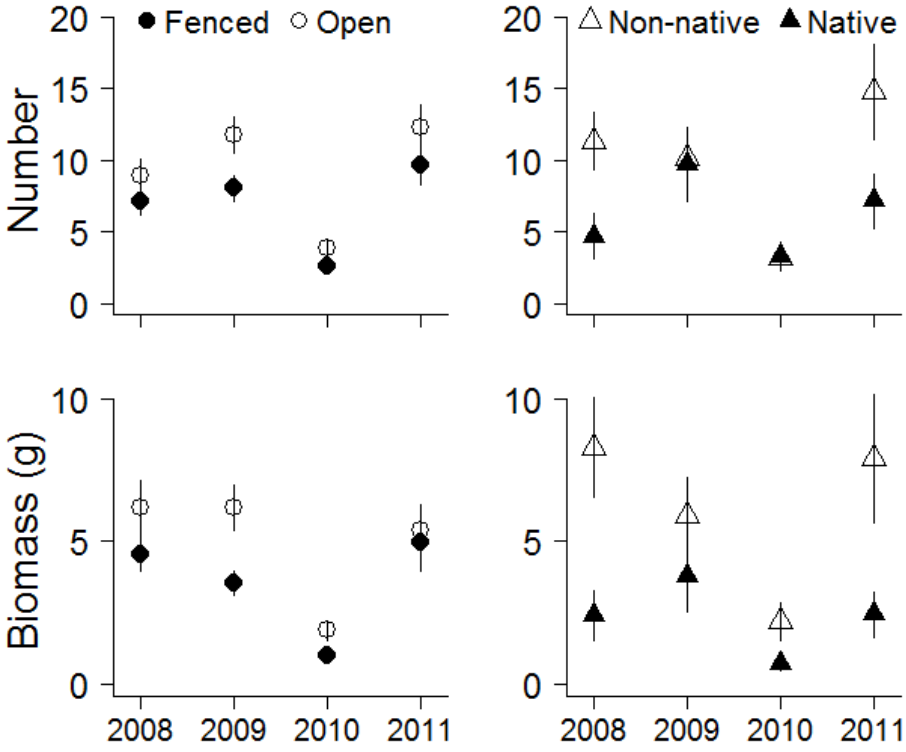

Fig. 26. Density (number per 0.25 m^{-2}) and biomass (g per 0.25 m^{-2}) of earthworms captured in fenced and open plots (left) and in plots dominated by native and non-native vegetation (right) at West Point, NY from 2008-2011. Fences were erected three weeks prior to 2008 earthworm sampling. Data are untransformed means ± 1 SE, N=12. Note different scales on number and biomass y-axes.

Earthworm biomass analyses indicated a significant effect of year, vegetation origin and leaf litter volume. Three models were included in the candidate set. Given the data, the best model included a polynomial effect of year, vegetation origin, leaf litter volume and an interaction between vegetation origin and sampling year and it had 1.39 times the explanatory

power than the next model which also included a fencing effect (**Table 18**). Biomass was lower in 2010 compared to the other sampling years; was on average 61% higher at sites dominated by non-native plants than at sites dominated by native vegetation and decreased with leaf litter volume (**Table 19**, **Fig. 26**). Similar to the effect of non-native plants on earthworm density, the effect of vegetation origin on biomass depended on sampling year: Earthworm biomass was higher at sites dominated by non-native plant species only in 2008 and 2011 (**Fig. 26**).

Table 19. Model results for the effects of vegetation origin, fencing and their interaction on earthworm density and biomass (g) at 12 sites at West Point, NY from 2008-2011.

Factor	Density			Biomass		
	Estimate	SE	t	Estimate	SE	t
Intercept	8.68	1.96	4.42	1.58	0.24	6.7
Y(L)	0.75	1.47	0.51	-0.33	0.13	-2.53
Y(Q)	6.39	1.47	4.34	0.52	0.13	3.91
Y(C)	5.43	1.47	3.69	0.41	0.14	3.03
VO	-3.65	2.67	-1.37	-0.69	0.34	-2.06
Fencing	2.35	1.08	2.18			
LLvol				-1.49	0.78	-1.91
VO x Y(L)	-0.53	2.08	-0.25	0.11	0.2	0.55
VO x Y(Q)	-6.95	2.08	-3.34	-0.39	0.19	-2.08
VO x Y(C)	-0.61	2.08	-0.29	0.08	0.19	0.45

L linear, Q quadratic, C cubic, LLvol leaf litter volume, VO vegetation origin, Y year

Analyses by genus indicated that density and biomass of *Amynthas* spp. and *Aporrectodea* spp. were correlated with year and site and by a polynomial effect of vegetation cover (*Amynthas* spp. only, **Table 20**, **Fig. 27**). *Lumbricus* spp. density and biomass also varied among sites and sampling years but they were significantly higher in open vs. fenced plots (**Table 20**, **Fig. 27**).

Table 20. Model selection results for linear model analyses of earthworm density and biomass at 12 sites at West Point, NY from 2008-2011. Only sites at which the genus was present were included in the analysis.

Genus	Density					Biomass				
	Model	K	AICc	ΔAICc	w_i	Model	K	AICc	ΔAICc	w_i
Amynthas	Y + S	7	156.93	0	0.67	Y + S + C	8	30.69	0	0.50
	Y + S + C^2	9	159.12	2.19	0.23	Y + S + C^2	9	30.88	0.19	0.45
Aporrectodea	S	5	143.16	0	0.94	Y	4	50.70	0	0.97
Lumbricus	Y + S/F	16	195.45	0	0.75	Y + S/F	16	109.16	0	0.85

C cover, F fencing; S site, Y year

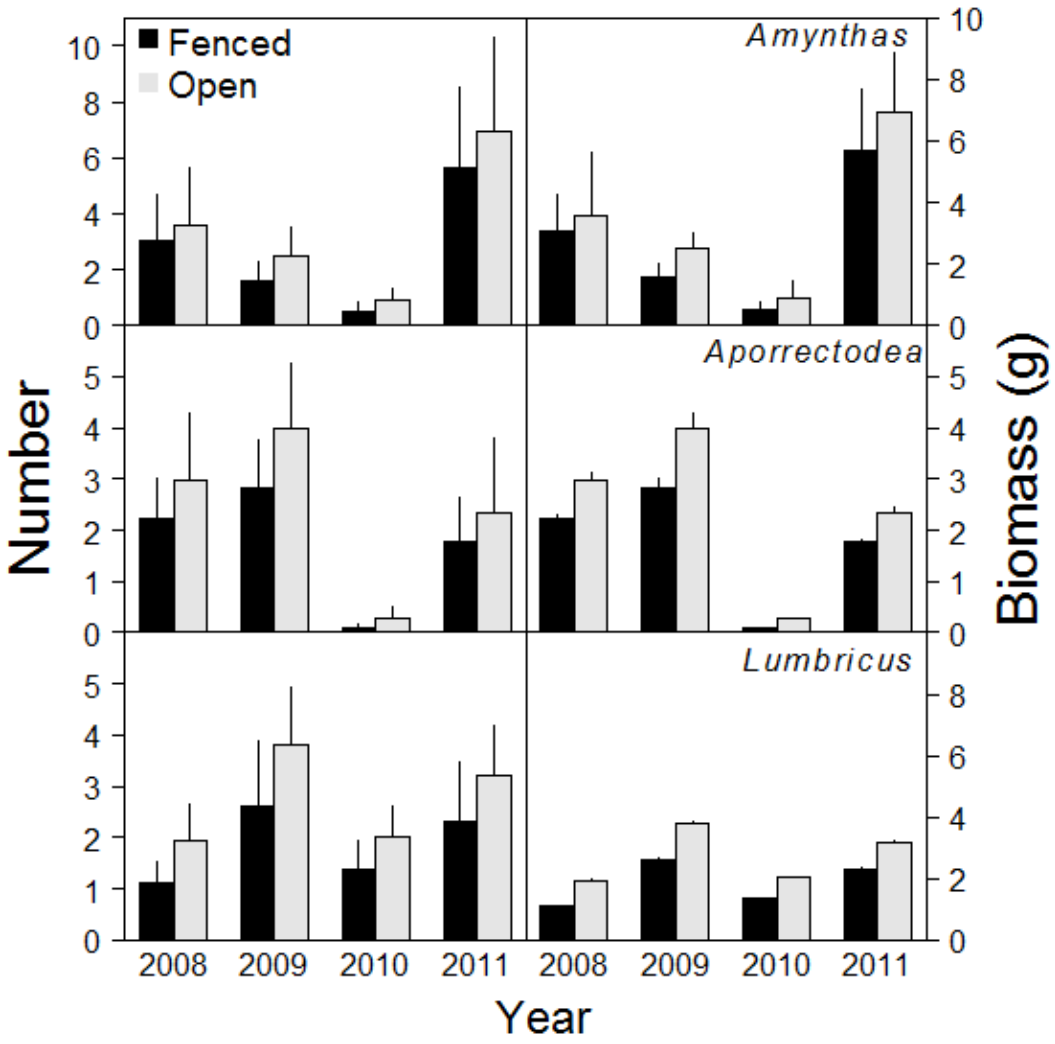

Fig. 27. Density (number per 0.25 m^{-2}) and wet biomass (g per 0.25 m^{-2}) of most abundant earthworm genera captured in fenced and open plots at West Point, NY from 2008-2011. Top panels correspond to *Amynthas*, middle panels to *Aporrectodea* and bottom panels to *Lumbricus*. Data are untransformed means ± 1SE, N=12. Note different scales on number and biomass y-axes. We excluded sites where the genus was not present or at very low density from all analyses.

Immature earthworms dominated the majority of sites, accounting for 60-97% of collected earthworms. The proportion of immature earthworms, evaluated through a GLMM with binomial errors, varied between years, decreased with leaf litter volume and was significantly higher at sites dominated by non-native plant species than at sites dominated by native species (estimate=-2.60 ± 0.62, z=-4.12, P<0.001, **Fig. 28**). The selected model (lowest AICc, *wi*=0.62) had three times more explanatory power than the next model, which also included an effect of fencing (ΔAICc=2.16, *wi*=0.21).

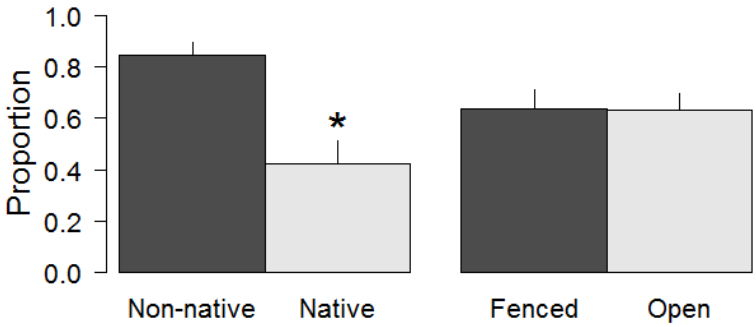

Fig. 28. Proportion of immature earthworms according to vegetation origin (non-native or native) and fencing (fenced or open plot) of earthworms captured at West Point, NY from 2008-2011. Data are means ± 2SE (N= 3 years).

3.3.4. Slug monitoring

Over the four-year study period we captured 1066 slugs at our 12 field sites at West Point NY, grouped into three species: *Arion subfuscus, Deroceras sp* and *Pallifera dorsalis* (A. Binney). The non-native *A. subfuscus* was the most abundant species, comprising 99% of captures. We only captured one individual of *Deroceras sp* and five *P. dorsalis* individuals. Slugs were absent (or nearly absent) from two study sites (Site 2 and 4), which both have low earthworm abundance and low cover of non-native plant species (see section 2.1 for a description of the study sites). Slug abundance was quite variable across our field sites in all years (possibly due to the small number of replicates; 5 per plot), with lowest captures recorded in 2011. Analyses indicated that slug captures and biomass were independent from fencing (factor was dropped from selected models), but were higher at sites with high earthworm density and at sites dominated by non-native plant species (significant year x non-native plant dominance interaction, **Table 21**, **Fig. 29**). Whether the simultaneous high abundance of non-native earthworms and non-native slugs reflects positive interactions between these two groups of organisms or is a function of plant community and site abiotic conditions is presently unclear.

Table 21. Model results for the effects of vegetation origin, fencing and earthworm density on slug captures and biomass (g) at 10 sites at West Point, NY from 2008-2010. We included site and plot within site as random factors in all models. We excluded data from Site 2 and 4 and from 2011 from all analyses.

Factor	Number			Biomass		
	Estimate	SE	T	Estimate	SE	t
Intercept	0.91	0.17	5.27	0.72	0.15	4.74
Year L	0	0.12	-0.03	-0.16	0.11	-1.41
Year Q	-0.8	0.12	-6.83	-0.67	0.11	-5.88
VO	0.77	0.26	2.96	0.71	0.23	3.1
EW	-0.4	0.32	-1.25	-0.35	0.28	-1.25
Y(L) x VO	0.37	0.18	2	0.22	0.18	1.22
Y(Q) x VO	0.77	0.18	4.17	0.68	0.18	3.73

EW earthworm density; VO vegetation origin

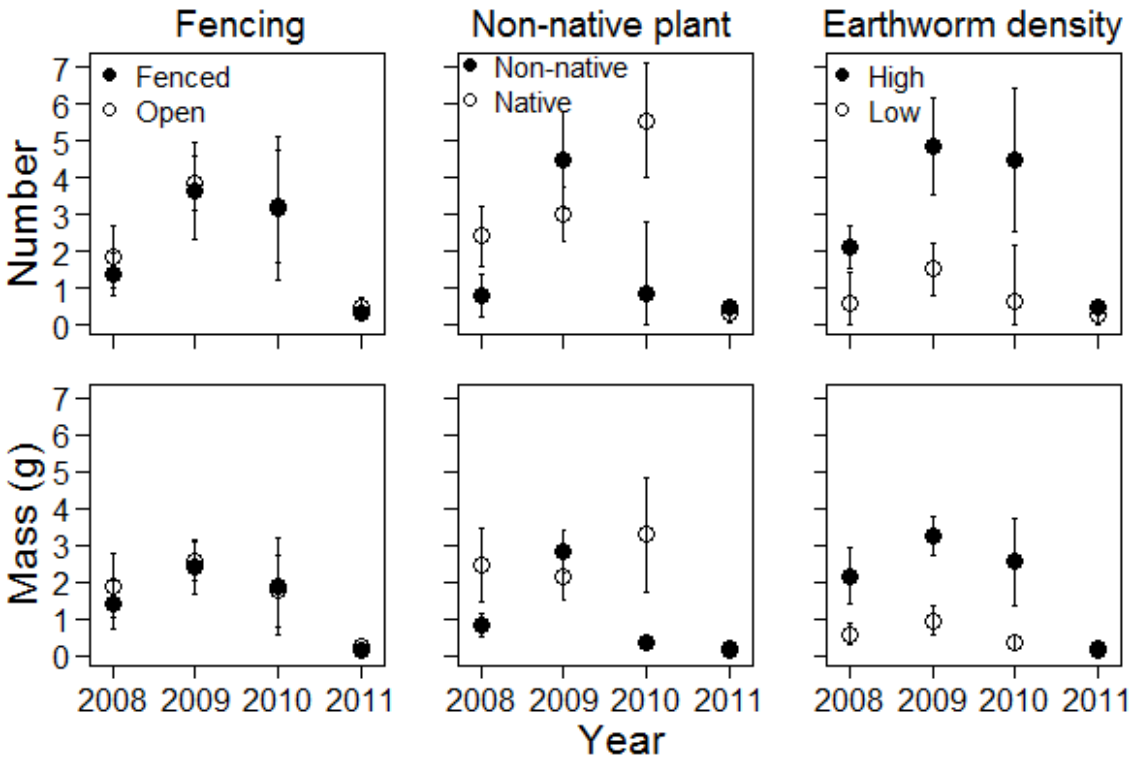

Fig. 29. Number and biomass (g) of slugs captured in fenced and open plots (left), at sites dominated by native and non-native vegetation (middle) and at sites with high and low earthworm density (right) at West Point, NY from 2008-2011. Data are untransformed means ± 1 SE, N= 5 samples per 12 fenced and open plots, 6 sites with native or non-native vegetation, and 4 and 8 sites with low and high earthworm density, respectively.

3.3.5. Root-weevil monitoring

Barypeithes pellucidus abundance was very patchy within sites (ranging from 0 to 53 in 2009, 0 to 18 in 2010, and 0 to 81 in 2011 in a single 0.25m^2 sample), and also varied widely among field sites and between years (data not shown). *Barypeithes pellucidus* was consistently absent from Sites 2 and 12 in all three years and our analyses, not surprisingly, indicated that neither presence of introduced plants nor fencing affected weevil presence or abundance. When all samples were included, the probability of *B. pellucidus* presence increased with leaf litter biomass (estimate= 0.004 ± 0.0004, z=8.645, P<0.001) and was higher in 2010 than in 2009 (estimate= 0.29 ± 0.07, z=83.89, P<0.001; **Fig. 30**). In fact, when only samples with *B. pellucidus* presence were examined, the relative abundance of these weevils was still positively correlated with leaf litter biomass (estimate= 0.001 ± 0.0004, z=4.43, P<0.001) although unaffected by year or fencing (P>0.05).

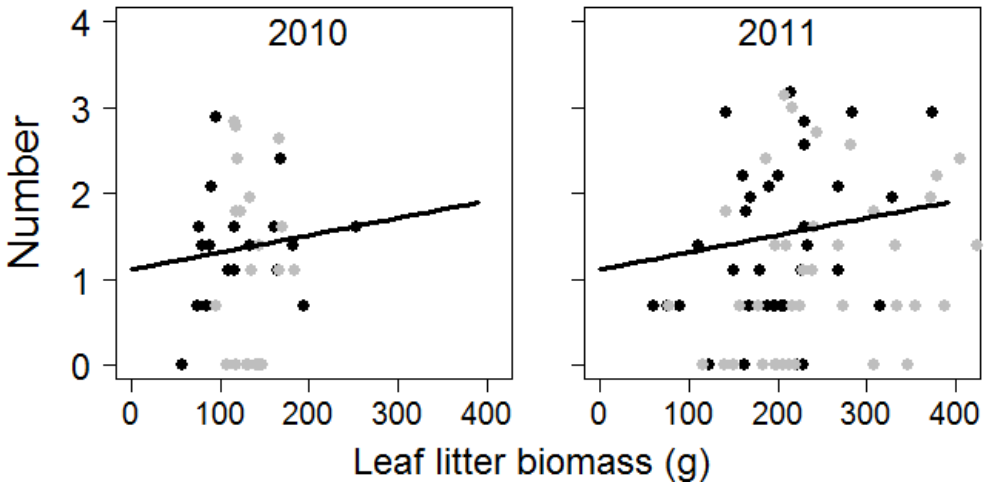

Fig. 30. *Barypeithes pellucidus* relative abundance as a function of leaf litter biomass in open (gray) and deer-fenced plots (black) at 12 sites at West Point NY in 2009 and 2010. Line represents GLMM with Poisson error predictions. Data is a subset of records where *B. pellucidus* were present only.

The relationship between *B. pellucidus* abundance and leaf litter biomass implies a potential interaction with earthworms, which reduce leaf litter. However, we did not see any relationship with worm abundance or species dominance at the plot level. The enormous variability in weevil abundance could be explained by aggregation pheromones or unknown site variables that are difficult to discern unless many more and more frequent samples can be collected. This was outside our financial and logistical possibilities. We are unable to greatly increase sample sizes in our plots since this introduces a disturbance (litter removal) that potentially affects other biota (earthworms, seeds) and processes (decomposition, nutrient cycling, water infiltration rates, etc.). Clearly the high abundance of weevils (and consequently of root feeding larvae) is expected to affect nutrient cycling and plant performance as we suggested in our proposal. At the level of resolution we are able to achieve in the field, we are unable to document this effect and will need to rely on our mesocosm experiments to address *B. pellucidus* impact on our target plant species (see below).

3.4. Reseeding experiment

Carex retroflexa germination in the field was extremely low: In 2011 we recorded only 20 seedlings germinated from 1920 seeds planted (0.01 %) in 2010. Only three of those seedlings survived over winter from 2011 to 2012 and by July 2012 all were absent. In 2012 we recorded two additional seedlings, which were still alive by the end of the growing season. *Carex retroflexa* germinated at 8 of the 12 study sites and the majority of seedlings were recorded in the open plots (15 out of 22, 68%). These poor germination results contrast with the results of germination trials conducted under controlled greenhouse conditions (section 2.7.1), where 73-77% of seeds germinated. We conclude that seed viability is not a problem for this species, and we speculate that micro-site availability and suitability in the field is likely driving this pattern. However, we also cannot exclude potential biological factors such as seed pathogens or other seed predators that may cause the low germination rates we encountered at our field sites.

In 2010, 273 *Aristolochia serpentaria* seedlings germinated from 1920 seeds planted (mean germination across sites of 14%) in 2009. Germination was higher under the non-native plant *M. vimineum* (29%) than at sites dominated by native vegetation (10%) or sites dominated by two other non-native species: *A. petiolata* (12%) and *B. thunbergii* (14%). In 2010, we did not find any differences in germination rates between open and fenced plots or among sites with low or high earthworm density.

One-year old *A. serpentaria* seedlings were impossible to separate from new emergents; therefore, our data reflect a conditional probability of seedling survival given that the seed survived and germinated (hereafter referred to as recruitment). Proportion of surviving recruits decreased with sampling year and survival continued to be consistently higher at sites dominated by *M. vimineum*. While recruitment was lower in 2010 and similar among sites that were not dominated by *M. vimineum* (native, *B. thunbergii* and *A. petiolata*), by 2012 recruitment at *A. petiolata* sites (which are also heavily dominated by the non-native earthworm *Amynthas* sp.) was significantly lower than recruitment at native and *B. thunbergii* sites (**Fig. 31**). The candidate model set included two models: The best model (*wi*=0.66) had 2.12 times the explanatory power of the next model (ΔAIC=1.53, *wi*=0.31) and included a year, vegetation type and fencing effect plus their interactions (except for a fencing x vegetation interaction, **Table 22**). The second model also included the fencing x vegetation interaction.

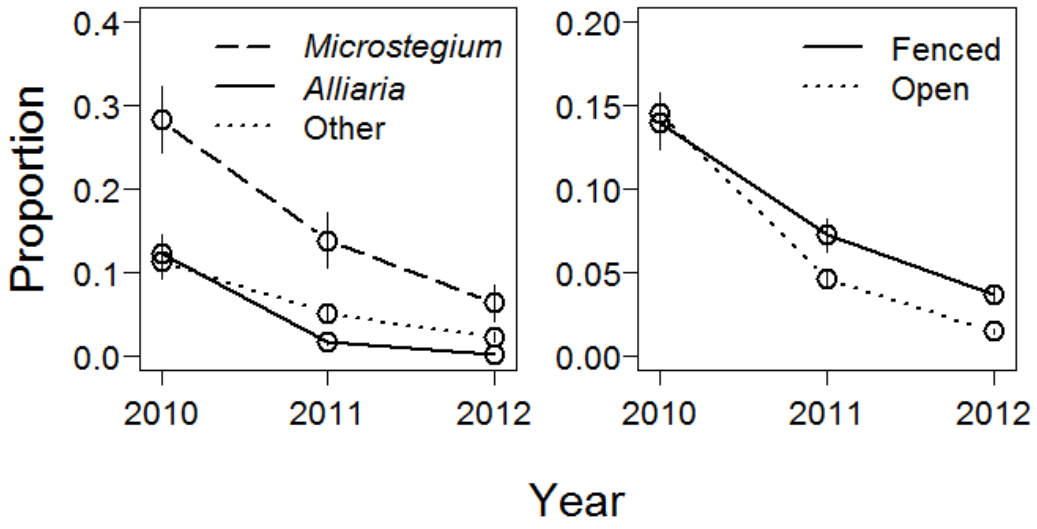

Fig. 31. *Aristolochia serpentaria* recruitment from seeds placed individually into vegetation dominated by either a focal non-native plant (*M. vimineum*, *A. petiolata*, *B. thunbergii*) or native species at 12 sites at West Point, NY. Category "Other" (left panel) refers to sites dominated by native species and by *B. thunbergii*. Data are means ± 2SE. Note the different scales between panels.

Table 22. Model results for the effects of vegetation origin and fencing on *A. serpentaria* recruitment from seeds placed individually into vegetation dominated by either a focal non-native plant (*M. vimineum, A. petiolata, B. thunbergii*) or native species at 12 sites at West Point, NY.

Factor	Estimate	SE	Z
Intercept	3757.37	791.87	4.74
Year	-1.87	0.39	-4.75
V-other	-2366.81	800.8	-2.96
V-M	-2254.94	817.71	-2.76
Fencing	906.69	315.36	2.88
Y x I-other	1.18	0.4	2.96
Y x I-M	1.12	0.41	2.76
Y x F	-0.45	0.16	-2.88

M *M. vimineum*; Other: native vegetation and *B. thunbergii*, Y year, V vegetation, F fencing.

Given the low number of surviving *A. serpentaria* seedlings, we analyzed seedling size with separate models for each year, such that site was not included as a random factor in any of the models. Also, we excluded *A. petiolata* sites, as no seedlings survived through 2012. ANOVA results in both years, indicated that seedlings were taller under *M. vimineum* than under native vegetation, but that there was no difference with *B. thunbergii* ($F_{2,29}$=5.67, P=0.008 in 2011 and $F_{2,14}$=4.7, P=0.02 in 2011; a posteriori Tukey tests; **Fig. 32**). In 2012 only, we found that seedlings growing under *M. vimineum* had more leaves than seedlings growing under native vegetation or *B. thunbergii* ($F_{2,14}$=7.29, P=0.006; **Fig. 32**). We found no differences in leaf width and no effect of fencing or earthworm density.

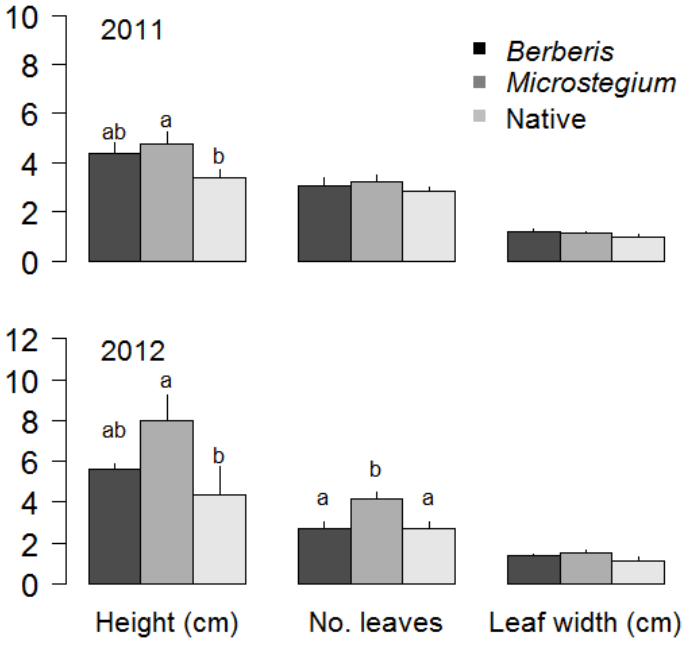

Fig. 32. Plant height (cm), number of leaves and leaf width (cm) of *A. serpentaria* seedlings recruited from seeds placed individually into vegetation dominated by either a focal non-native plant (*M. vimineum*, *A. petiolata*, *B. thunbergii*) or native species at 12 sites at West Point, NY in 2011 (top) and 2012 (bottom). Data are means + SE. Letters represent a posteriori Tukey comparisons.

Trillium erectum seedlings emerging from our planted seeds were first recorded in 2011, with mean germination across sites of 19% from 1920 seeds planted in 2009. In 2012 we recorded five additional seedlings. Given the low number of new seedlings in 2012, we combined the 2011 and 2012 datasets to explore the effect of fencing, vegetation origin and earthworm density on total germination across both years. Model selection results identified three candidate models, with the best model having 2.2 times the explanatory power of the next best model (**Table 23**). Similar to *A. serpentaria*, germination of *T. erectum* was higher under the non-native plant species *M. vimineum* than at sites dominated by native vegetation or by the non-native species *A. petiolata* and *B. thunbergii* (**Fig. 33, Table 24**). We did not find any differences between open and fenced plots or among sites with low or high earthworm density.

Table 23. Model selection results for GLMM with binomial error analyses of *T. erectum* germination and cotyledon survival. Site and plot within site were included as random factors in all models.

Variable	Model	K	AICc	ΔAICc	w_i
Germination	V (Bt, Other, Mv)	5	308.36	0	0.44
	V (Bt, Ap, Other, Mv)	6	309.93	1.57	0.20
	V (Bt, Ap, Other, Mv) + F	7	310.13	1.77	0.18
Survival	E + V x F	7	433.15	0	0.45
	E + V + F	6	434.07	0.92	0.29
	E + V	5	434.82	1.67	0.20

Ap *A. petiolata*, Bt *B. thunbergii*, F fencing, Mv *M. vimineum*, Other remaining vegetation types, V vegetation type.

Table 24. Model results for the effects of vegetation origin on *T. erectum* germination and overwinter survival.

Variable	Factor	Estimate	SE	z
Germination	Intercept	-3.02	0.44	-6.79
	V-other	1.36	0.49	2.79
	V-Mv	2.50	0.58	4.27
Survival	Intercept	-1.21	0.36	-3.34
	Ew	1.35	0.43	3.16
	V – M	2.48	0.63	3.92
	F	-0.29	0.43	-0.68
	F x V	-1.63	0.86	-1.89

Ew earthworm density, F fencing, Mv *M. vimineum*; Other: native vegetation and *A. petiolata*, V vegetation.

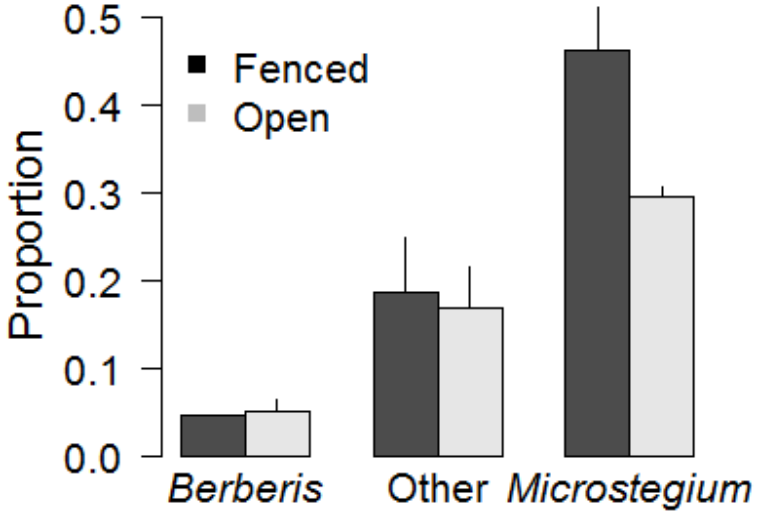

Fig. 33. Germination (proportion) of *T. erectum* seeds placed individually into vegetation dominated by either a focal non-native plant (*M. vimineum*, *A. petiolata*, *B. thunbergii*) or native species at 12 sites at West Point, NY. Category "Other" refers to sites dominated by native species and by *A. petiolata*. Data are means + 2SE.

Survival of *T. erectum* germinants from 2011 to 2012 averaged 43% across sites. It was significantly higher at sites with low earthworm density and at sites dominated by the non-native *M. vimineum* (**Fig. 34**). The best model (**Table 23**) also indicated a minor interaction between fencing and vegetation type (**Table 24**): Survival in the fenced plots at sites dominated by *M. vimineum* was higher than survival in the open plots.

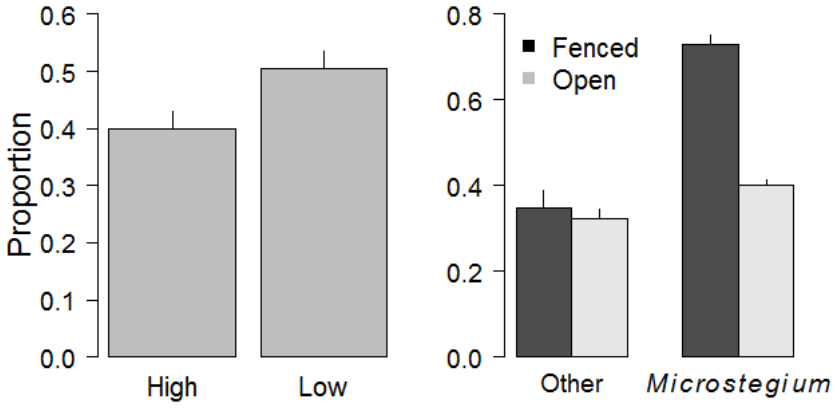

Fig. 34. Effects of earthworm density (left) and of vegetation type and fencing (right) on survival of *T. erectum* germinants planted as seed into 12 sites at West Point, NY. Category "other" refers to sites dominated by native vegetation and by focal non-native species: *A. petiolata* and *B. thunbergii*. Data are means + 2SE, N=4 and 8 sites with low and high earthworm density, respectively; and N=10 and 2 sites with vegetation type "other" and *M. vimineum*, respectively.

3.5. Transplant experiment

3.5.1. Survival

Agrimonia rostellata survival from plantings in 2011 to August 2012 averaged 56% across all sites (25% to 90% survival). Four models were included in the candidate set (**Table 25**) and they all included an interactive effect between slug exclusion and vegetation origin or fencing. The best model had 1.52 times the explanatory power than the next best model, which also included earthworm density. Nutrient addition was not included in any model. When we excluded slugs, through molluscicide application, juvenile survival decreased at sites dominated by non-native vegetation and at open plots, but not at sites dominated by native vegetation or at fenced plots (**Table 26**, **Fig. 35**). Leaf length at planting was positively correlated with survival, but did not differ across treatments or sites (P>0.05).

Table 25. Model selection results for GLMM with binomial error analyses of survival of *A. rostellata, A. serpentaria* and *T. erectum* juveniles transplanted into open and fenced plots at 12 sites at West Point, NY. Site and plot within site were included as random factors in all models.

Genus	Density				
	Model	K	AICc	ΔAICc	w_i
Agrimonia rostellata	Length + S*(F + VO)	9	570.94	0	0.29
	Length + E + S*(F + VO)	10	571.84	0.90	019
	S*(F + VO)	8	572.28	1.34	0.15
	S*VO	6	572.36	1.41	0.14
Aristolochia serpentaria	~1	3	622.89	0	0.46
	VO*N	6	623.11	0.21	0.42
Trillium erectum	F + N + Ew	6	659.27	0	0.32
	F + N + Ew*S	8	660.25	0.97	0.20
	F + Ew	5	660.36	1.08	0.19
	F + N + Ew + S	7	660.91	1.63	0.14

E earthworm density, F fencing, N nutrient addition, S slug, VO vegetation origin

Aristolochia serpentaria survival ranged from 20% to 88% with mean survival across sites of 56%. Two models were included in the candidate set (**Table 25**). The best model only included the random terms (intercept estimate=0.29 ± 0.25, z=1.25) and had 1.1 times the explanatory power of the next model, which included an interaction between vegetation origin and nutrient addition. According to the second best model, survival of plants that did not receive nutrients was higher at sites dominated by native vegetation (61% vs. 49%). Leaf width at planting had no effect on survival and did not differ across treatments or sites (P>0.05).

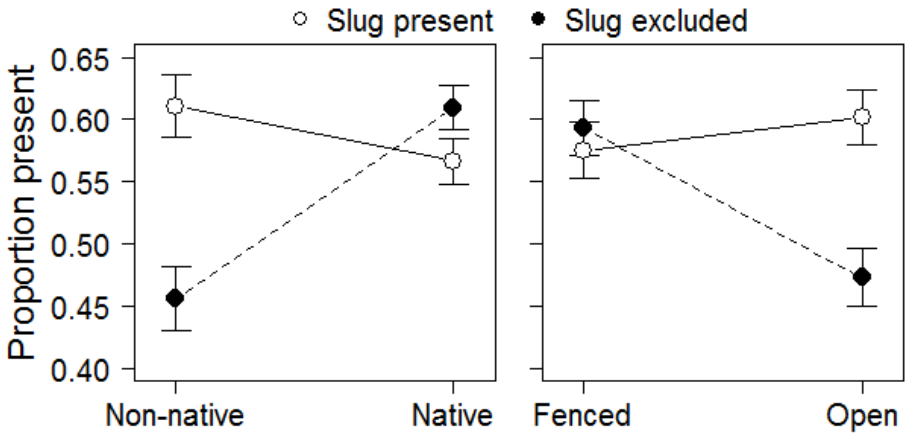

Fig. 35. Interactive effects of slug exclusion with vegetation origin (left) and fencing (right) on survival of *A. rostellata* seedlings transplanted into 12 sites at West Point, NY. Data are means ± SE of 40 plants per site, N=6 sites per vegetation type.

Carex retroflexa survival ranged from 20% to 98% across the 12 sites (mean 65%). Survival of this species was best explained by the random model only (*wi*=0.59, intercept estimate=0.83 ± 0.42, z=2.13). Almost 9% of *C. retroflexa* seedlings were dug up, presumably by rodents. Removal was particularly high at the open plot of one of the sites dominated by the non-native *M. vimineum*, where 55% of seedlings were dug up (11 out of 20 planted). Nutrient addition increased the likelihood of being dug up at two sites where 5 out of the 6 seedlings that were dug up received nutrients.

Table 26. Model results for the effects of vegetation origin, fencing, slug exclusion, earthworm density and their interaction on survival of *A. rostellata* and *T. erectum* juveniles transplanted into 12 sites at West Point, NY.

Species	Factor	Estimate	SE	z
Agrimonia rostellata	Intercept	-0.89	0.76	-1.18
	Leaf length	0.25	0.13	1.88
	S	0.55	0.38	1.45
	F	-0.75	0.51	-1.48
	VO	0.89	0.72	1.25
	S*F	0.91	0.43	2.09
	S*VO	-1.25	0.44	-2.85
Trillium erectum	Intercept	0.19	0.19	1.01
	F	-0.43	0.19	-2.33
	Nutrient	-0.33	0.19	-1.77
	Ew	0.61	0.23	2.62

Ew earthworm density, F fencing, S slug exclusion, VO vegetation origin

Trillium erectum survival ranged from 30% to 78% across sites (mean survival 50%). Four models were included in the candidate set: the best model had 1.6 times the explanatory

power of the next model (**Table 25**) and included a positive effect of fencing, a negative effect of nutrient addition and a negative correlation with earthworm density (**Table 26**, **Fig. 36**). The nutrient effect was minor (P=0.07). Slug exclusion and its interaction with earthworm density were included in the remaining models.

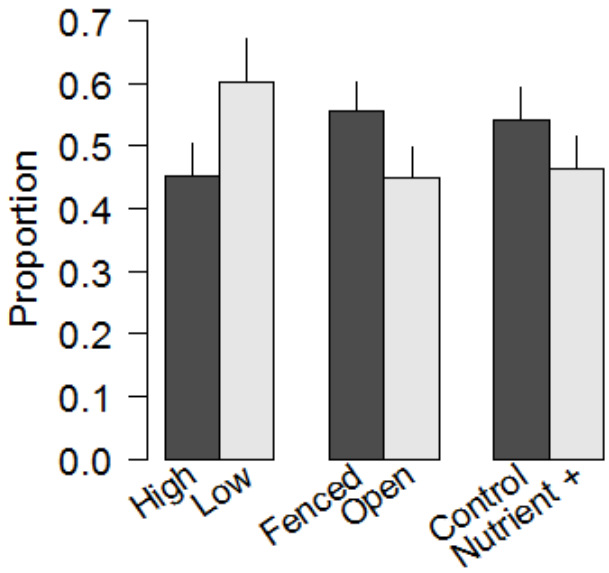

Fig. 36. Effects of earthworm density, fencing and nutrient addition on survival of *T. erectum* seedlings transplanted into 12 sites at West Point, NY. Data are means + 2SE of 40 plants per site, N=4 and 8 sites with low and high earthworm density, respectively.

3.5.2. Seedling growth

Agrimonia rostellata seedlings grew over the course of the study: Average leaf length increased from 3.9 ± 0.04 cm at planting to 8.96 ± 0.19 cm in August 2012. The best model for leaf length included only a positive effect of time and no effect of any study factor. Plant height at the end of the study was slightly higher at sites dominated by non-native vegetation (12.42 ± 0.78 cm at non-native sites vs. 9.48 ± 1.16 cm at native sites; estimate= 2.14 ± 1.01, t=2.1), but we found no difference among the remaining study factors.

Aristolochia serpentaria seedlings grew taller with wider leaves over the course of the study (**Fig. 37**). Leaf width was best explained by two models which included a positive effect of time (estimate=1.43E-03 ± 7.5E-05, t=19.08) and nutrient addition (estimate=1.04 ± 2.23E-02, t=4.66), a negative relationship with earthworm density (estimate=-1.12 ± 2.21E-02, t=-5.08) and an interaction between time x earthworm density (estimate=7.43-E4 ± 1.45-04, t=5.12). The two models differed on the inclusion of a time x nutrient interaction (ΔAIC=1.25, *wi* =0.65 and 0.35, respectively). Plant height was also positvely correlated with time (estimate=1.13E-02 ± 2.94E-04, t=38.23) and nutrient addition (estimate=4.34 ± 1.04, t=4.19), but plants were significantly shorter at sites dominated by native vegetation (estimate=-0.25 ± 0.1, t=-2.28). Only one model was included in the candidate set (*wi* =0.69) and it had 3.45 times more explanatory power than the next model (ΔAIC=2.48).

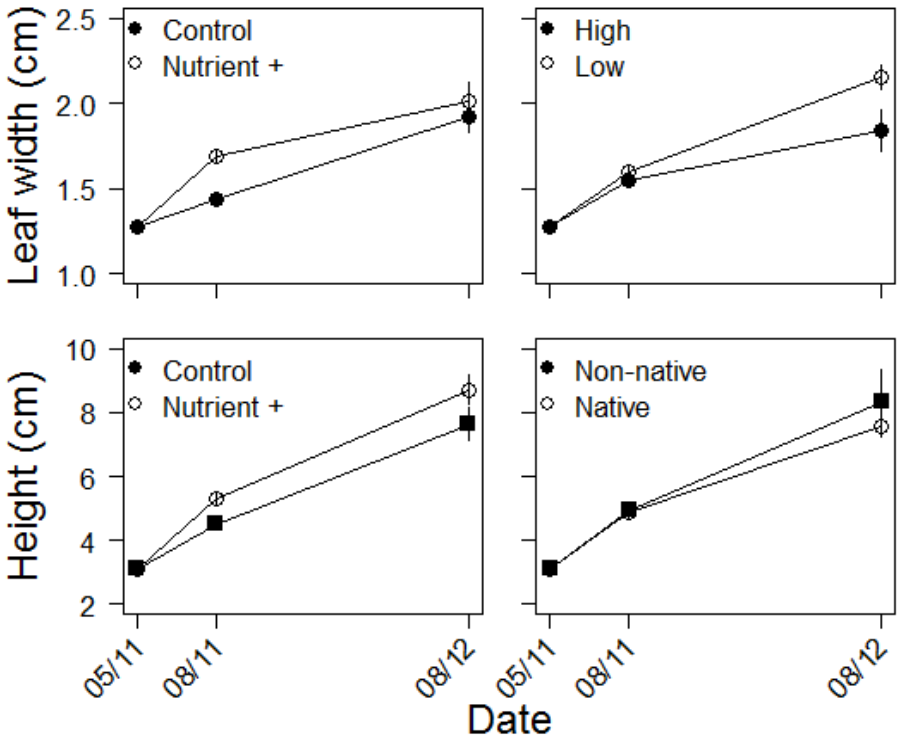

Fig. 37. Effect of time, nutrient addition, earthworm density and vegetation origin on leaf width (top panels) and height (bottom panels) of *A. serpentaria* seedlings transplanted into 12 sites at West Point, NY. Data are means ± SE of 40 plants per site, N=4 and 8 sites with low and high earthworm density, respectively.

Carex retroflexa seedlings grew over the course of the study from an average of 3.09 ± 0.024 culms per plant at planting to 11.15 ± 0.51 culms per plant in October 2012 (**Fig. 38**). Culm number was explained by only model which included a positive effect of time (estimate= 0.02 ± 0.001, t=16.95), nutrient addition (estimate= -6.15 ± 1.95, t=-3.16) and earthworm density (estimate= 13.5 ± 2.04, t=6.75), as well as interaction terms between both factors and time (estimate= -9.1E-04 ± 1.3E-04, t=-6.82 for nutrient addition, and estimate= 4E-04 ± 1.3E-04, t=3.2 for earthworm density).

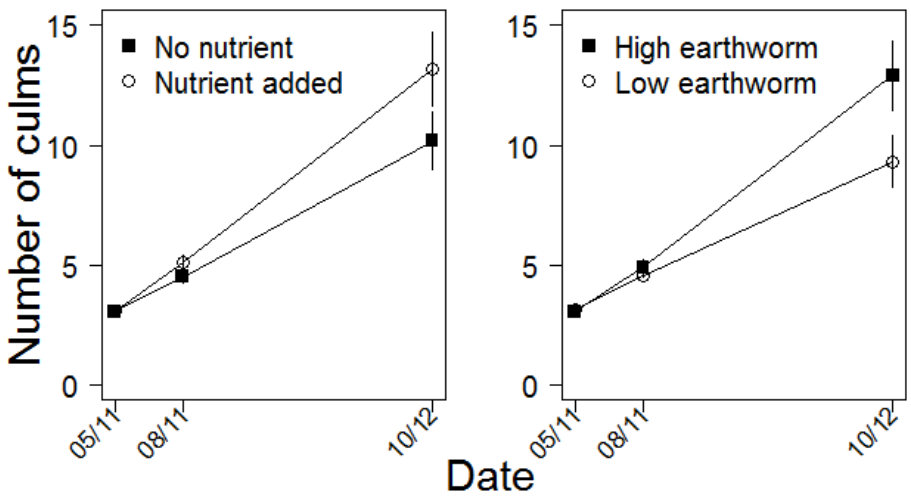

Fig. 38. Effect of time, nutrient addition and earthworm density on number of culms of *C. retroflexa* transplanted into 12 sites at West Point, NY. Data are means ± SE of 40 plants per site, N=4 and 8 sites with low and high earthworm density, respectively.

Leaf width (cm) of *T. erectum* seedlings that did not transition from a 1-leaf to a 3-leaf stage increased slightly after one year of planting (95% CI for leaf width at planting: 1.64-1.75 cm vs.: 1.73-2.0 cm in May 2012). When plants transition from a 1-leaf to a 3-leaf stage, the individual leaflets are narrower but the plant effectively triples its leaf area. Fifteen percent of plants transitioned from a 1-leaf to a 3-leaf stage and two individuals transitioned into a 2-leaf stage. Mean leaf width at the time of planting of individuals that did transition to a multiple leaf stage was slightly above the overall mean (2.06 ± 0.11 vs. 1.7± 0.03; $F_{1,478}$=18.51, P<0.001). Leaf width and the probability of transition from 1-leaf to 3-leaf were not affected by any of the study factors.

We found a significant effect of *T. erectum* maternal line on leaf width difference between the start and termination of the experiment ($F_{21,146}$=2.94, P<0.001). Post-hoc Tukey tests (alpha=0.95) indicated that only a few maternal lines differed, namely the two maternal lines that showed the highest growth (R and S) were different from maternal lines C, I and O (**Fig. 39**). However it is important to note that the experiment was unbalanced due to differential propagation success of the maternal lines. Transition to a 3-leaf stage occurred in 10 of the 23 maternal lines.

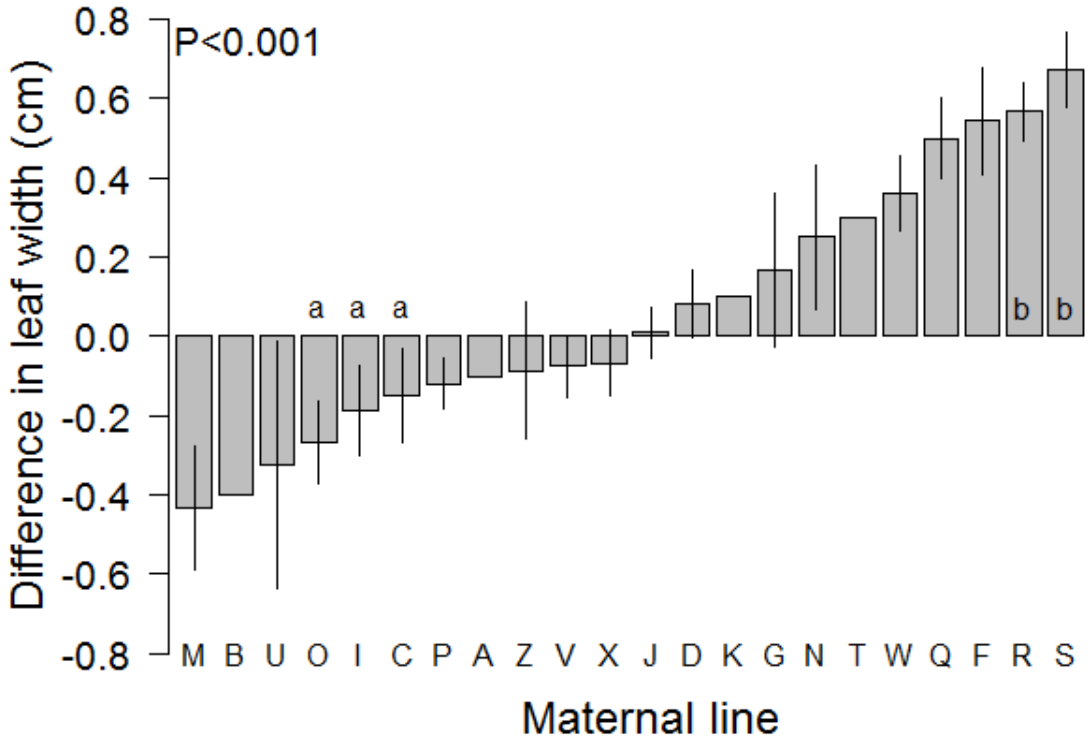

Fig. 39. Difference in leaf width (cm) from 2012 to 2011 according to maternal line of *T. erectum* seedlings planted at 12 sites at West Point, NY. Data are means ± SE. Number of plants varied according to maternal line and ranged from 3 to 40. No error bars indicate N<3. Only individuals that did not transition to a multiple leaf stage were included in the analysis. Different letters indicate significant differences of the mean (Tukey test).

3.5.3. Reproductive output

Two of the four SAR species flowered: *A. rostellata* and *C. retroflexa*. *Agrimonia rostellata* flowered at 9 of the 12 study sites with proportion of plants flowering ranging from 0.05 to 0.40 (N=20 plants per site, overall 15% of live plants flowered). The best model (*wi*=0.65) had 1.97 times the explanatory power than the next model (ΔAICc=1.35, *wi*=0.33) and included a positive effect of plant height (estimate=0.46 ± 0.08, z=6.05) and weaker but positive effect of fencing (estimate=-1.0 ± 0.55, z=-1.81) on the probability of flowering (**Fig. 40**). Slug exclusion and nutrient addition had no effect. We did not test for earthworm density and plant origin, as *A. rostellata* only flowered at a subset of the sites. Given the low proportion of flowering plants, we did not formally analyze the variation in number of flowers (mean per plant: 10.1 ± 1.52). Nevertheless, data indicate that the number of flowers tends to be lower at sites with low earthworm density and when slugs are excluded.

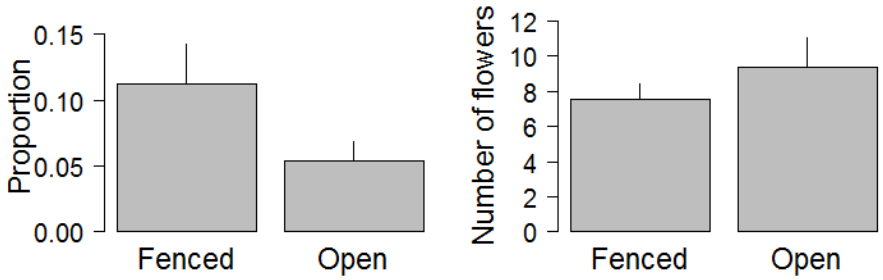

Fig. 40. Proportion of flowering plants (left) and of number of flowers (right) produced by *A. rostellata* transplanted at fenced and open plots at 12 sites at West Point, NY. Data are means ± 2SE of 12 sites.

Carex retroflexa flowered at 10 of 12 sites with proportion of flowering plants ranging from 0.025 to 0.75 per site (mean: 0.16 ± 0.23). We recorded the highest proportion of flowering *C. retroflexa* at a site dominated by native graminoids (see section 3.3.1 for plant community descriptions). *Carex retroflexa* flowering probability was best explained by one model (*wi*=0.71, ΔAICc=3.33) which included a positive effect of earthworm density and a significant interaction between nutrient addition and earthworm density (**Table 27**, **Fig. 41**). Fertile *C. retroflexa* produced on average 5.83 ± 0.63 flowering culms per plant with 5.47 ± 0.62 seeds per culm. The proportion of flowering:vegetative culms was best explained by one plausible model (*wi*=0.74, ΔAICc=2.16), which indicated a higher proportion of flowering culms at sites with high earthworm density, an interaction between nutrient addition and earthworm density, and an interaction between nutrient addition and slug exclusion (**Table 27**, **Fig. 41**).

Table 27. Model results for the effects of nutrient addition, earthworm density, slug exclusion and vegetation origin and their interaction on *C. retroflexa* probability of flowering, ratio of flowering to vegetative culms and on the number of viable seeds produced per flowering culm.

Variable	Factor	Estimate	SE	T
Probability of flowering	Intercept	-1.69	0.59	-2.86
	Nutrient addition	0.46	0.39	1.19
	Earthworm	-2.91	1.42	-2.05
	Nutrient x Earthworm	2.92	1.2	2.43
Ratio flowering:vegetative	Intercept	-1.23	0.2	-6.22
	Nutrient	-0.29	0.2	-1.43
	Earthworm	-2.25	1.07	-2.11
	Slug exclusion	-0.41	0.22	-1.86
	Nutrient x Earthworm	2.69	1.05	2.55
	Nutrient x Slug	0.8	0.27	3.01
Seeds per flowering culm	Intercept	4.4	1.08	4.07
	Total culm number	0.14	0.03	4.19
	Vegetation origin	-2.51	0.91	-2.76
	Earthworm	-2.54	1.14	-2.23

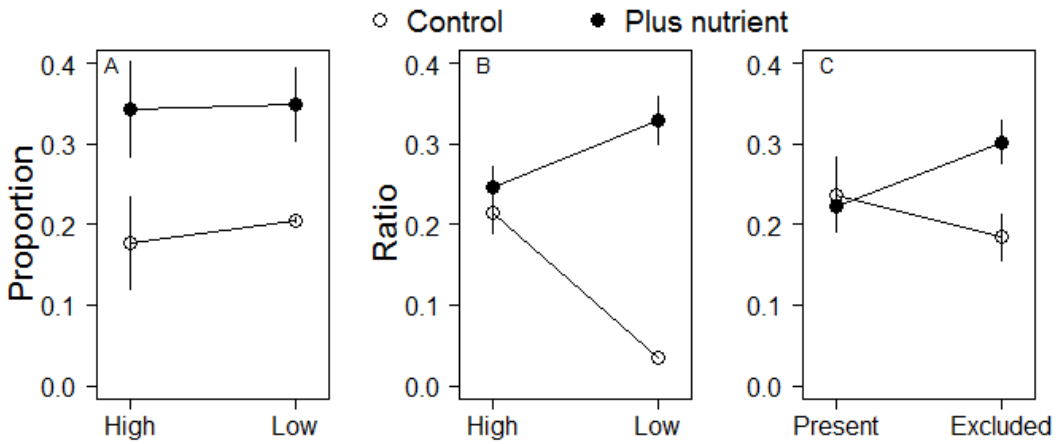

Fig. 41. Interactive effects of nutrient addition, earthworm density (A and B) and slug exclusion (C) on proportion of flowering culms (A) and ratio of flowering: vegetative culms (B and C) of surviving *C. retroflexa* transplanted at 12 sites at West Point, NY in spring 2011 and measured in June 2012. Data are means ± 2SE. Note that *C. retroflexa* flowered at 6 and 4 sites with high and low earthworm density, respectively.

The number of seeds per flowering culm was best explained by one plausible model (*wi*=0.72, ΔAICc=2.37), which indicated higher seed production with increasing number of total culms and higher seed production at sites dominated by non-native vegetation and at sites with high earthworm density (**Table 27**, **Fig. 42**). Overall, all measures of reproductive output (flowering, proportion of flowering to vegetative culms and seed production) where positively influenced by high earthworm density; however, we found important interactions between this factor, nutrient addition and slug exclusion.

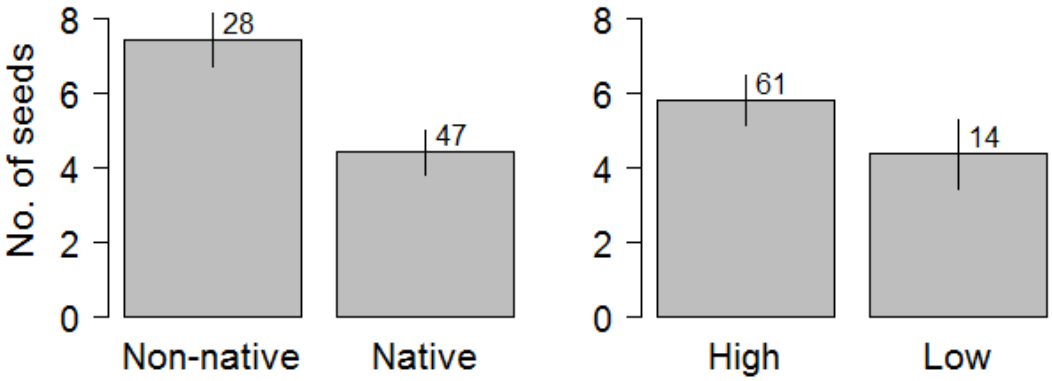

Fig. 42. Number of seeds per culm according to vegetation origin (left) earthworm density (high N=8 sites, low N=4 sites) produced by *C. retroflexa* planted at 12 sites at West Point, NY. Data are means ± 2SE, N=4 and 6 for non-native and native sites, N=6 and 4 for sites with high and low density, respectively. Numbers on bars indicate the number of flowering plants.

3.6. Plant mark-recapture

3.6.1. *Agrimonia rostellata*

We marked and followed 50 *A. rostellata* individuals in the fenced plot and 50 individuals in the open plot from 2009-2012. The majority of individuals were present throughout the study, except for five individuals from the open plot that failed to emerge in 2012 (one of those last emerged in 2010). Short of digging them up, it is now impossible to determine if these individuals died or entered dormancy. Deer-fencing effectively reduced browsing of this species, but the rate at which browsing decreased was different between study years (**Table 28, Fig. 43**). We recorded browsing inside the fenced plot, which was attributed to pre-fence construction browse (2009) and to small rodent and possible insect herbivory (2010 – 2011). No browse was recorded in the fenced plot in 2012.

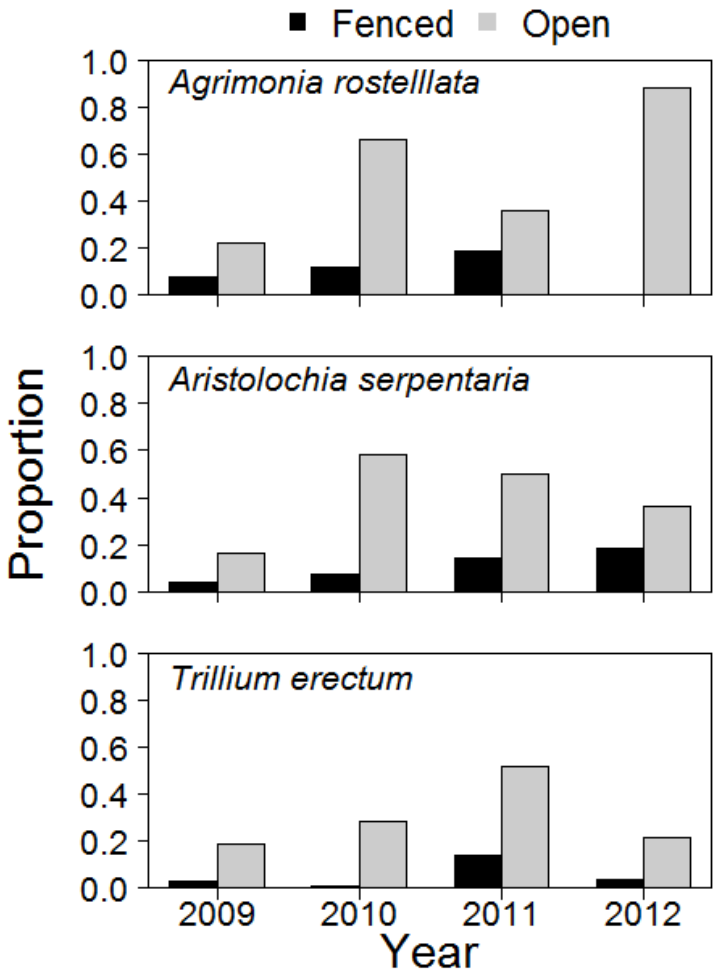

Fig. 43. Proportion of *A. rostellata* (A), *A. serpentaria* (B) and *T. erectum* (C) plants attacked by herbivores in one open and fenced plot per species from 2009-2012. Data are means of 80-176 individuals/species. Data for 2009 reflect a fence effect of 2 months (fences were erected mid-June 2009).

Table 28. Model results for GLMM with binomial errors analyses of the effects of fencing and year on browsing probability. Plant identity was included as a random factor.

Factor	*Agrimonia rostellata*		*Aristolochia serpentaria*		*Trillium erectum*	
	Estimate ± SE	z	Estimate ± SE	z	Estimate ± SE	z
Fencing	2.70 ± 0.37	7.35***	1.92 ± 0.30	6.33***	2.19 ± 0.31	7.04***
Year (L)	-0.85 ± 0.75	-1.15	0.83 ± 0.29	2.88**	0.42 ± 0.26	1.60
Year (Q)	-1.57 ± 0.63	-2.45*	0.98 ± 0.27	-3.66***	-0.94 ± 0.25	-3.80***
Year (C)					-0.82 ± 0.23	3.58***
F x Y(L)	2.54 ± 0.82	3.13**	NS	NS	NS	NS
F x Y(Q)	1.80 ± 0.71	2.53*	NS	NS	NS	NS

*P<0.05, **P<0.01, ***P<0.001, NS not significant
L linear, Q quadratic, C cubic, F fencing, Y year

Analysis of *A. rostellata* plant size indicated that after five years of fencing, protected plants grew taller with more and longer leaves (**Table 29, Fig. 44**), and differences between fenced and plant exposed to deer herbivory increased over time and became quite apparent in 2012 (significant year x fencing interaction). Flowering probability did not differ between the open and fenced plot (P>0.05, **Fig. 45**), but fertile plants in the fenced plot produced more flowers (**Table 29, Fig. 44**).

Table 29. Model results for LMM analyses (GLMM with Poisson errors for number of flowers) of the effects of fencing and year on *A. rostellata* stem height, number of leaves, leaf length and number of flowers. Plant identity was included as a random factor in all models.

	Stem height			No. of leaves			Leaf length			No. of flowers		
	ES	SE	t	ES	SE	T	ES	SE	t	ES	SE	z
Year(L)	10.34	1.24	8.35	5.49	0.17	32.88	2.69	0.28	9.68	0.74	0.07	11
Year(Q)	-0.21	1.22	-0.18	1.47	0.16	9.26	0.61	0.27	2.24	-0.10	0.05	-1.75
Year(C)	-3.20	1.21	-2.65	0.49	0.16	3.17	-1.25	0.27	-4.61			
Fencing	-6.06	1.87	-3.25	-0.11	0.15	-0.74	-1.50	0.46	-3.28	-0.63	0.18	-3.45
Y(L) x F	-11.82	1.72	-6.86	-1.09	0.24	-4.63	-3.83	0.39	-9.91	-0.82	0.20	-4.14
Y(Q) x F	-3.58	1.71	-2.09	-1.69	0.22	-7.65	-0.73	0.38	-1.89	-0.80	0.13	-6.12
Y(C) x F	-0.84	1.7	-0.49	-0.54	0.22	-2.47	0.32	0.38	0.85			

ES estimate, L linear, Q quadratic, C cubic, F fencing, Y year

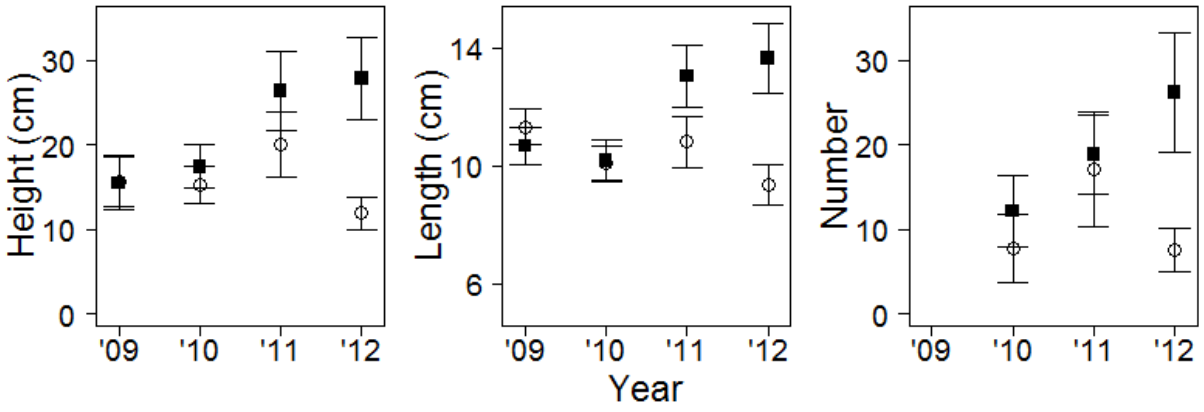

Fig. 44. Plant height (cm), leaf length (cm) and number of flowers of *A. rostellata* adult individuals in open and fenced plots in 2009-2012. Data are means ± 2SE of 50 individuals per plot. Note different scales on each panel.

The probability of flowering increased with sampling year (estimate=1.64 ± 0.50, z=3.30, P<0.001) and it was positively correlated with plant height (estimate=0.38 ± 0.04, z=8.59, P<0.001; **Fig. 45**), measured to the base of the inflorescence. Given the influence of plant height on flowering probability we used the median height of nonflowering plants (10.78 cm) as a break point to separate small vs. large plants in the demographic model (section 2.11).

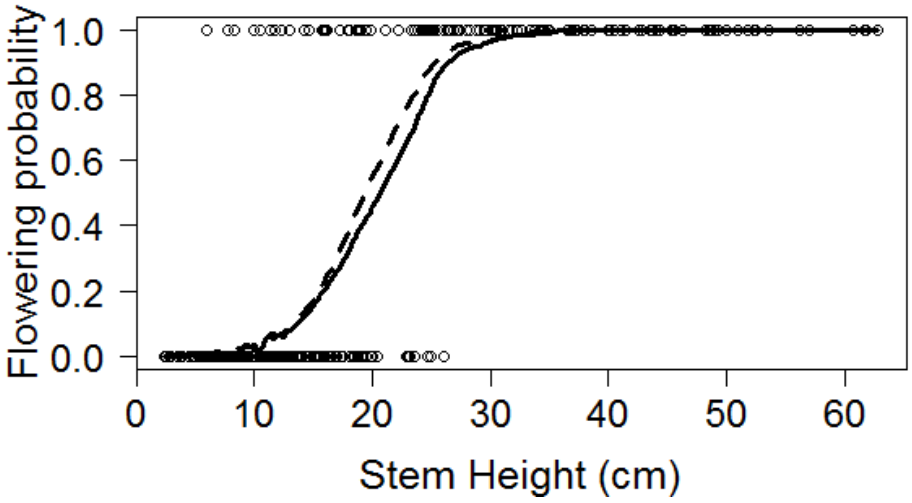

Fig. 45. Effect of stem height (cm) on probability of flowering of *A. rostellata* measured at open (dashed line) and fenced (continuous line) plots at one site in 2009-2012. Lines depict GLMM predictions.

3.6.2. *Aristolochia serpentaria*

From 2009-2012 we marked and followed 53 individuals in the fenced plot and 54 individuals in the open plot. Over the course of the study, six plants (three per plot) were dormant for one year and emerged the following year, three plants (one in the fenced and two in the open plot) did not emerge 2011-2012, and a surprisingly high number of individuals did not emerge in 2012 (15 plants in the fenced plot and 9 plants in the open plot). The year 2012 was exceptionally dry and warm at West Point potentially explaining the increased frequency of dormancy. Given the current data, it is impossible to know if these plants died or entered dormancy. However, observed dormancy in previous years indicates that dormancy is a plausible strategy for this species. We would like to understand the conditions under which *Aristolochia* enters dormancy and whether dormancy provides a benefit or cost for the population. Plant demography models seldom include dormancy as a stage, partly because it is unobservable, and therefore difficult to quantify. We will need to conduct long term monitoring of the marked plants, such that, in the future, we can include dormancy as a stage in our demography model. We have arranged with the natural resources branch at West Point to continue our investigations into additional years, which should provide us with the desired information on dormancy or mortality. We will build new models as additional information can be integrated.

Fencing effectively reduced deer browsing of this species, although plants were still attacked by rodents and insects (**Table 28, Fig. 43**). Analysis of *A. serpentaria* plant size indicated that after five years of fencing plants grew taller with more and wider leaves (**Table 30, Fig. 46**). Height differences appear to exist from the onset of the study, but remaining size measurements clearly indicate that plants were larger in the fenced plot.

Table 30. Model results for LMM analyses of the effects of fencing and year on *A. serpentaria* stem height, number of leaves and leaf length. Plant identity was included as a random factor in all models.

	Stem height			No. of leaves			Leaf length		
	ES	**SE**	**t**	**ES**	**SE**	**t**	**ES**	**SE**	**t**
Year(L)	0.15	0.44	0.35	0.94	0.18	5.10	5.18	0.14	38.18
Year(Q)	-0.43	0.43	-1.02	0.35	0.18	1.98	0.49	0.15	3.20
Year(C)	-0.44	0.41	-1.08						
Fencing	-3.79	0.60	-6.29	-1.26	0.18	-7.01	-0.18	0.15	-1.23
Y(L) x F	-0.93	0.61	-1.52	-0.95	0.25	-3.72	-1.10	0.19	-5.76
Y(Q) x F	1.33	0.59	2.24	0.15	0.25	0.59	-0.72	0.21	-3.41
Y(C) x F	1.32	0.58	2.29						

ES estimate, SE standard error, L linear, Q quadratic, C cubic, F fencing, Y year

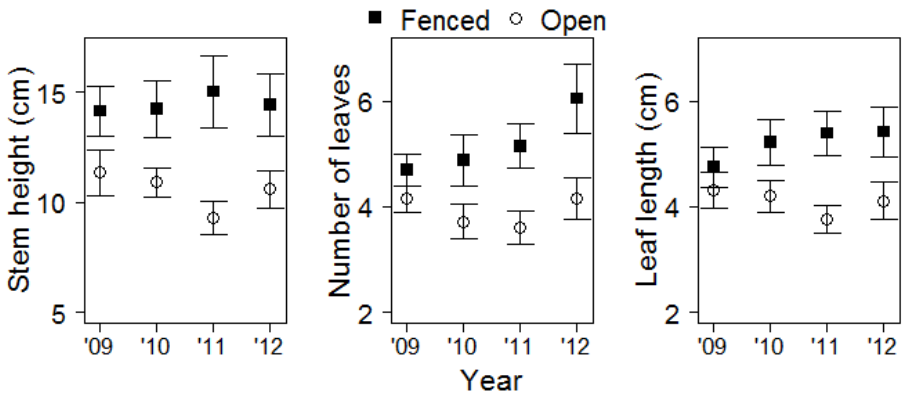

Fig. 46. Plant height (cm), number of leaves and leaf length (cm) of *A. serpentaria* adult individuals at one open and one fenced plot at West Point NY in 2009-2012. Data are means ± 2SE of 107 individuals. Note different scales on each panel.

Aristolochia serpentaria probability of flowering increased significantly with leaf length (estimate= 1.14 ± 0.22, z=5.11, P<0.001) and varied across years (P<0.05 for linear, quadratic and cubic polynomial terms, and for interaction terms), presenting an extremely low flowering probability in 2012 (**Fig. 47**). Leaf width, leaf length and plant height were good predictors of plant fertility, but leaf length had the highest explanatory power. Therefore, median leaf length of sterile plants (4.3 cm) was used as a break point to separate small vs. large vegetative plants in the demographic model (section 2.11).

Although we did not find an effect of fencing on the probability of flowering, a higher proportion of plants flowered in the fenced plot in 2011 (**Fig. 47**, right). Given the low probability of flowering in 2012, we ran a separate model excluding 2012 data. In that case, we found a significant interaction between year and fencing, indicating no differences in 2009-2010 but a higher probability of flowering in the fenced plot in 2011 (fencing x year(Q) interaction: estimate= -1.76 ± 0.80, z=-2.19, P=0.02).

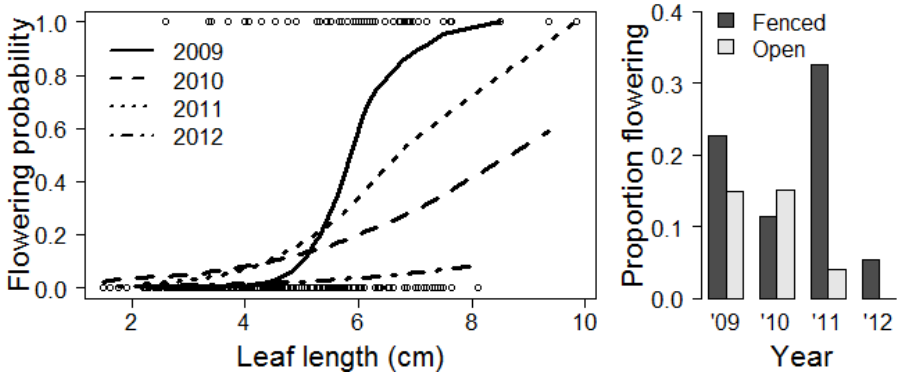

Fig. 47. Effect of leaf length (cm) and year on *A. serpentaria* probability of flowering (left) and proportion of *A. serpentaria* plants that flowered in open and fenced plots at West Point, NY from 2009-2012 (right). Lines depict GLMM predictions.

3.6.3. *Carex retroflexa*

From 2009-2012 we marked and followed 40 individuals in the fenced plot and 40 individuals in the open plot. All marked plants in the fenced plot were present throughout the study, but in the open plot one individual was absent since 2010 and apparently dead, and two individuals were lost (tags removed and plants could not be relocated). Apparently, mammals do not browse this species, since we did not detect any mammal browsing. *Carex retroflexa* size, measured as the number of culms (counted in November) varied with year (P<0.001) and was affected by a fencing x year interaction (P<0.001): After two years of fencing the total number of culms tended to be higher in the open than in the fenced plot (**Fig. 48**). Vegetative growth, measured as the ratio of new to old culms per plant was higher in the open plot from the onset of the study (estimate=0.096 ± 0.017, t=5.6) and varied with year (linear estimate=0.16 ± 0.01, t=14.37, quadratic estimate=-0.13 ± 0.01, t=-11.58). A second model that included an interaction between year and fencing also received support from the data (ΔAICc=0.19, AICc *wi*=0.42 vs. 0.46 for the model without interaction, **Fig. 48**).

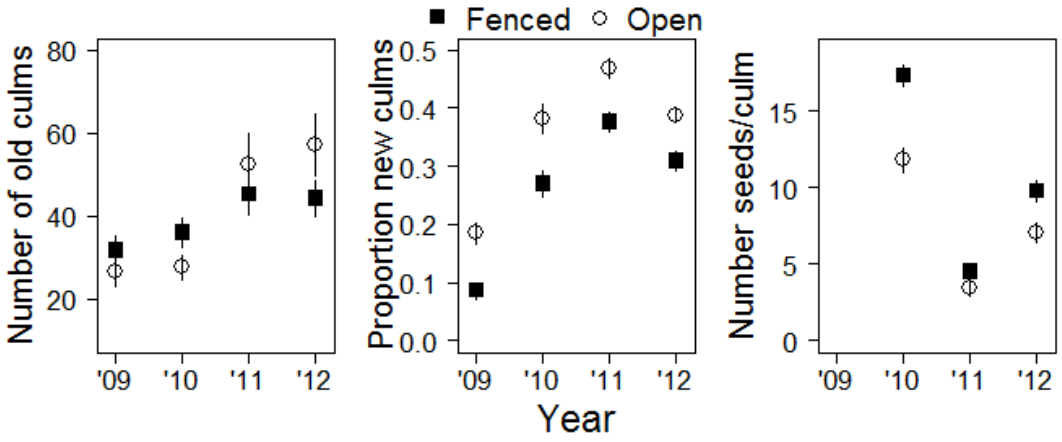

Fig. 48. Number of old culms in November (left), proportion of new to old culms in November (middle) and mean number of seeds per culm in June (right) of *C. retroflexa* individuals at open and fenced plots at New Paltz NY in 2009-2012. Data are means ± 2SE of 37-40 individuals/plot. Note different scales on each panel.

The number of flowering culms in June was best explained by one candidate model (AICc *wi*=1) which indicated a positive effect of the total number of vegetative culms in November of the previous year, a positive effect of fencing and significant differences between transitions (2009-10, 2010-11 or 2011-12) on flower culm production. The best model included all second level interactions, except for a fencing x number of old culms interaction (**Table 31**). While in 2009-2010 flower culm production was higher in the fenced plot, there were no differences in the next two transitions (**Fig. 49**). Further, flower culm production in 2011 was extremely low and in this year it was not positively correlated with number of old culms. The average number of seeds per culm (counted in June) was higher in the fenced plot (estimate= =3.09 ± 0.59, t=-5.19), but significantly lower in 2011 compared to other years (**Fig. 48**). Weather conditions may have affected sexual reproduction of this plant, which had significantly fewer flowering culms and number of seeds per culm under the drought conditions experienced in summer 2011. However, weather reports from the NE Regional Climate Center, hosted by

Cornell University, seem to indicate rather average precipitation in June and July 2011. Overall, results indicate a trend for vegetative reproduction to be more prevalent in the open plot (higher production of new culms), whereas sexual reproduction is stronger in the fenced plot.

Table 31. Model results for GLMM with Poisson error analyses of the effects of transition years, fencing and number of old vegetative culms in the previous year on *C. retroflexa* number of flowering culms. Plant identity was included as a random factor.

Factor	Estimate	SE	z
Intercept	2.68	0.09	28.53***
T1: 2010-11	-1.19	0.08	-14.06***
T2: 2011-12	0.17	0.06	2.9***
Fencing	-0.66	0.14	-4.74***
Culms L	8.35	0.67	12.44***
Culms Q	-2.42	0.64	-3.76***
T1: Fencing	-0.1	0.15	-0.65
T2:Fencing	1	0.09	11.59***
T1:Culms	-9.39	1.52	-6.16***
T2:Culms	1.47	0.58	2.53*
T1:Culms L	-2.31	1.93	-1.2
T2:Culms Q	1.29	0.58	2.22*

*P<0.05, **P<0.01, ***P<0.001, NS not significant
L linear, Q quadratic, SE standard error, T transition

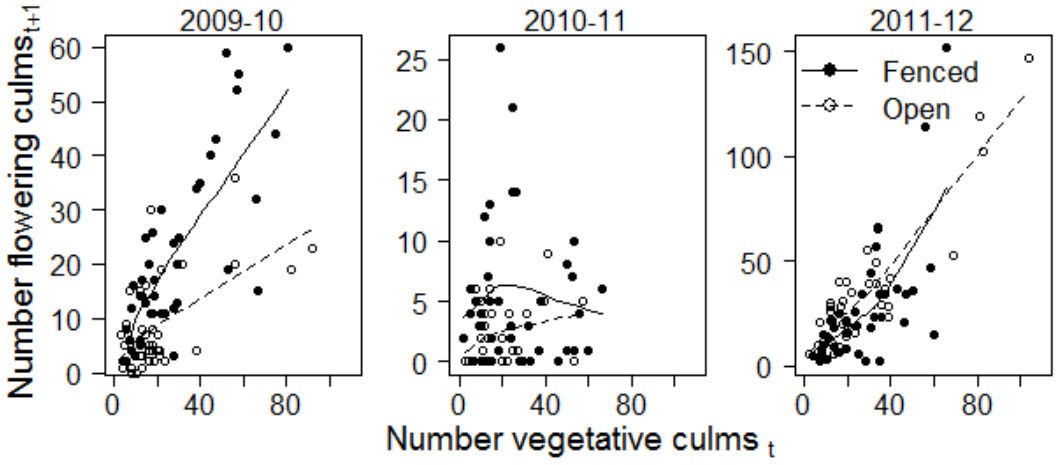

Fig. 49. Number of flowering culms in June as a function of the number of vegetative culms in November in the previous calendar year of *C. retroflexa* in open and fenced plots at New Paltz, NY. Lines depict GLMM with Poisson error predictions.

3.6.4. *Trillium erectum*

From 2009-2012 we marked and followed 97 individuals in the fenced plot and 87 individuals in the open plot. The majority of marked individuals (120) were present throughout the study, but several were dormant for one or two years (21 and 2 dormant individuals, respectively), or were absent since 2010 or 2011. Frequency of dormant and absent plants was similar between fenced and open plots. Plants inside the fenced plot experienced less browsing (**Table 28, Fig. 43**) and responded positively to protection from deer herbivory. By 2012, plants were significantly taller with wider leaves, and produced more seed in the fenced vs. open plot (**Table 32, Fig. 50**)

Table 32. Model results for LMM analyses (GLMM with Poisson errors for number of seeds) of the effects of fencing and year on *T. erectum* stem height, leaf width and number of seeds. Plant identity was included as a random factor in all models.

	Stem height			Leaf width			Number of seeds		
	ES	**SE**	**T**	**ES**	**SE**	**t**	**ES**	**SE**	**z**
Year(L)	-1.78	0.36	-4.98	0.4	0.17	2.42	-0.38	0.03	-13.04
Year(Q)	-1.85	0.36	-5.13	-0.08	0.17	-0.45	-0.01	0.02	-0.51
Year(C)	1.45	0.37	3.96	1.02	0.17	6.01	-0.59	0.21	-2.86
Fencing	-3.36	0.73	-4.62	-1.75	0.36	-4.87			
Y(L) x F	-2.72	0.52	-5.19	-1.67	0.24	-6.86	-0.6	0.08	-7.23
Y(Q) x F	1.02	0.56	1.81	-0.09	0.26	-0.33	-0.23	0.09	0.09
Y(C) x F	-0.06	0.61	-0.1	-0.23	0.28	-0.82			

ES estimate, SE standard error, L linear, Q quadratic, C cubic, F fencing, Y year

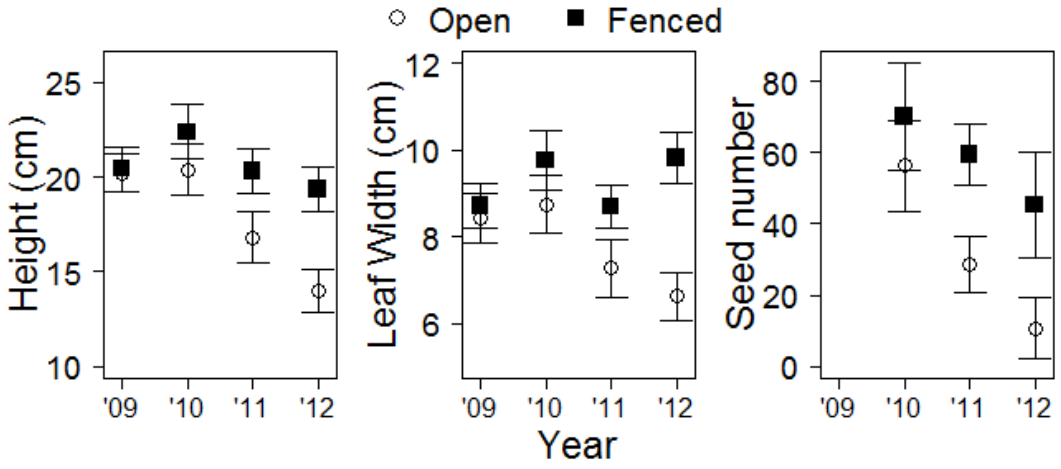

Fig. 50. Plant height (cm), leaf width (cm) and number of seeds of *T. erectum* adult individuals at open and fenced plots at Richford NY in 2009-2012. Seed number was recorded from a subset of fruits in 2009, and from all fruits in 2011 and 2012. Data are means ± 2SE. Note different scales on each panel.

Flowering probability of *T. erectum* plants increased with leaf width (estimate=-1.38 ± 0.15, z=9.08, P<0.001), was not affected by sampling year (P>0.05) and was significantly lower in the open plot vs. fenced plot (estimate=-1.84 ± 0.51, z=-3.55, P<0.001; **Fig. 51**) Similar to its congener *T. grandiflorum*, we found that leaf width in *T. erectum* was a good predictor of plant fertility and therefore we used the median leaf width of nonflowering three-leaf plants (5.4 cm) as a break up point to separate small from large vegetative plants in the demographic model (section 2.11).

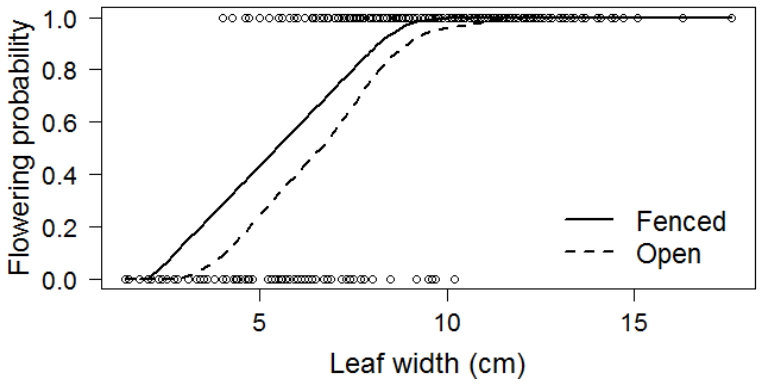

Fig. 51. Effect of leaf width (cm) and fencing on the probability of flowering of *T. erectum* measured at open (dashed line) and fenced (continuous line) plots at one site near Richford NY in 2009-2012. Lines depict GLMM predictions.

3.7. Common garden experiments

3.7.1. Slug palatability tests

3.7.1.1. Slug feeding trials

Seedling consumption varied across plant species ($F_{22,58}$=2.46, P=0.003), but was not affected by plant origin ($F_{1,58}$=0.09, P=0.76, **Fig. 52**). Consumption varied among slug species ($F_{2, 58}$= 8.47, P<0.0005) with the non-native *Arion subfuscus* and *Limax* maximus L. and the native *Deroceras laeve* (Müller) having similar but higher consumption than the native *Philomycus* sp (**Fig. 53**, top). Overall consumption by non-native slugs was slightly higher than consumption by native species ($F_{1,58}$=3.76, P=0.06).

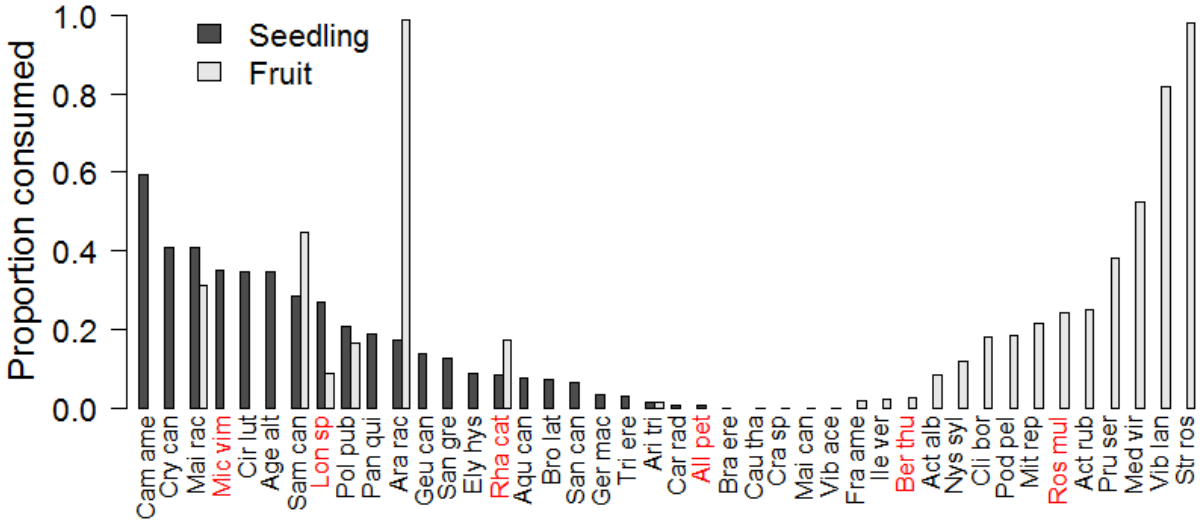

Fig. 52. Seedling and fruit consumption (proportion) of native (black) and non-native (red) plant species by four slug species of native and non-native origin. Plant species are sorted according to proportion of seedling and fruit consumed and are abbreviated as the first three letters of the genus followed by the first three letters of the species epithet. See **Table 5** for full plant species names.

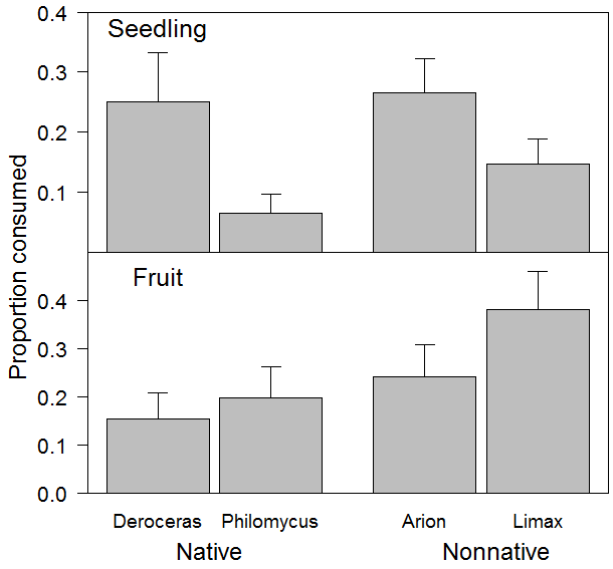

Fig. 53. Seedling (top) and fruit (bottom) consumption (proportion) as a function of slug origin and species.

Fruit consumption depended on plant species identity ($F_{21, 63}$=5.79, P<0.001), but not on plant origin (**Fig. 52**). Fruit consumption was significantly influenced by fruit color ($F_{2, 63}$=31.18, P<0.001), with purple fruits being more consumed (66%) than white-cream colored fruits (4%). Other fruit colors (red, black, blue) experienced intermediate and similar consumption of

approximately 20%. Overall, non-native slugs consumed a higher proportion of fruits than native slug species ($F_{1, 63}$=13.47, P<0.001) and consumption varied according to species ($F_{2, 63}$=3.75, P=0.03; **Fig. 53**). The small purple fruits of *Aralia racemosa* were highly favored and equally consumed by non-native (100%) and native (98.5%) slugs. In general, non-native and native slugs showed similar preferences for fruits of the tested plant species but differed in the amount of fruit consumed. Only one native plant species, *Mitchella repens*, was consumed more by native than non-native slugs (40% and 3%, respectively). All other fruits were consumed more by non-native slugs, or were equally refused by all slugs.

Eliasomes of the three *Trillium* species tested were readily consumed by native and non-native slugs (**Fig. 54**). Consumption was higher for *T. grandiflorum* and *T. undulatum* than for *SAR T. erectum* ($F_{2,27}$=4.08, P=0.03), but was not affected by slug origin or slug species (P>0.05 for all cases).

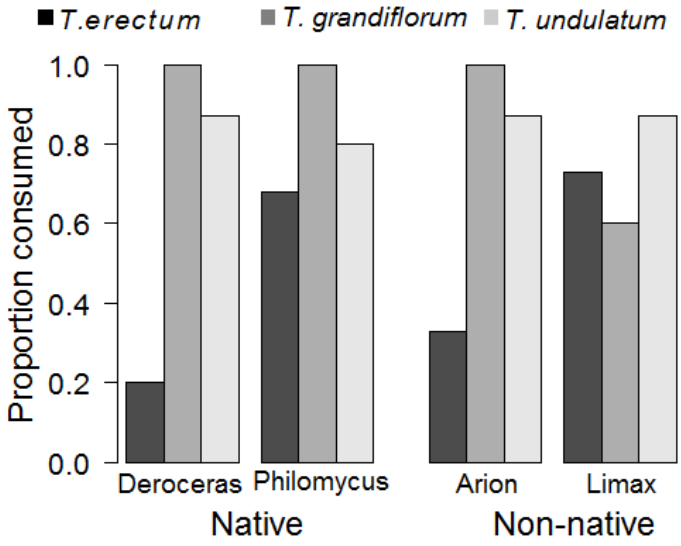

Fig. 54. Eliasome consumption (proportion) over a 4 d period of *Trillium erectum, T. grandiflorum* and *T. undulatum* according to slug origin and species.

3.7.1.2. *Trillium erectum* and *T. grandiflorum* slug preference and germination trial

Analyses of eliasome and fruit consumption, indicated one plausible model (ΔAICc>2 for all candidate models), which included a significant effect of time, plant tissue (fruit or eliasome) and slug treatment. Consumption increased with time and it was significantly higher when slugs were present (note that consumption refers to amount of tissue lost, by feeding and/or decomposition). While consumption of both species was similar, the lipid-rich eliasomes were highly preferred over fruits (**Fig. 55**). Further, we detected a significant interaction between time and organ: Eliasomes were completely consumed earlier than fruits, but by the end of the feeding trial individual seeds had been extracted out of the fruit and their eliasomes were consumed. More fruit than eliasome material disappeared from the control terrariums (significant organ x treatment interaction) and percentage disappearance in the control did not change with time (significant time x treatment interaction). We recorded germination in 45% of

planted fruits and it was significantly higher when fruits had been exposed to slugs (60% vs. 30%, in slug and control terrariums, respectively; Estimate = -1.25 ± 0.55, z = -0.23, P = 0.02), Interestingly, a higher proportion of fruits were moldy in the controls (0.23) than in the slug treatment (0.03) and moldy fruits tended to have a lower probability of germination, suggesting that slugs may benefit germination by decreasing fruit decay.

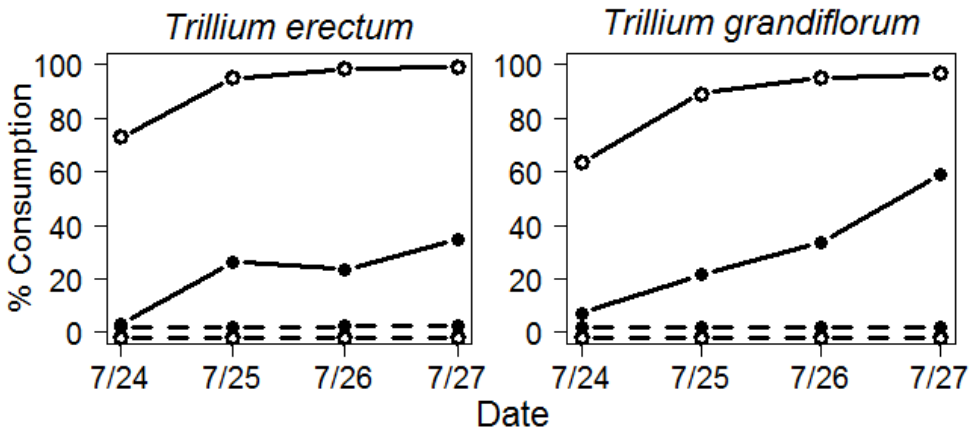

Fig. 55. Eliasome and fruit consumption (%) of *T. erectum* (left) and *T. grandiflorum* (right) by *Arion subfuscus* (solid lines) over a 5d period. Open circles represent eliasomes, filled circles represent fruits. Control treatments (dashed lines) at bottom of each panel represent decomposition only in absence of *A. subfuscus* (jittered to allow visualization).

3.7.1.3. Feeding trial and molluscicide effectiveness

Slug survival was significantly reduced when slugs were exposed to molluscicide: All slugs from no-molluscicide terrariums survived, whereas only 4% of slugs exposed to molluscicide survived. Analysis of consumption probability indicated two plausible models in the candidate set: the best model included plant species and treatment (slug-molluscicide and control treated as one level) as significant factors and it had 2.5 times the explanatory power of the next model, which separated slug-molluscicide and control treatments into two levels (**Table 33**). The control had no slug or molluscicide addition. Molluscicide addition effectively reduced the probability of consumption by 90%. While different plant species had a different probability of consumption, consumption was consistently highest in the slug-no molluscicide treatment (no treatment x plant species interaction), and did not differ between the control (no slug- no molluscicide) and the slug-molluscicide treatment (**Fig. 56**). We also found no effect of block. Slugs did not consume three species (*A. petiolata, C. radiata* and SAR *C. retroflexa*) over the 8 d study and slugs consumed only one individual of SAR *A. serpentaria*. Slug consumption of SAR *A. rostellata* was significantly higher than of the common congener *A. gryposepela*. Interestingly, both *Agrimonia* grown from seed species experienced herbivory in the control (no slug - no molluscicide) terrariums, while *A. rostellata* grown from tissue-culture did not. Slug consumption of *A. rostellata* was similar regardless of propagation method (tissue culture and grown from seed). Consumption of SAR *T. erectum* varied by stage: One-leaf seedlings (one year old) had twice the consumption rate (60%) compared to cotyledon stage seedlings (7 weeks old).

Table 33. Model selection results for logistic regression analysis of consumption probability and LM of percent consumption of 13 plant species by *Arion subfuscus* in presence and absence of molluscicide.

Candidate models	K	AICc	ΔAICc	w_i
Consumption probability				
Treatment[a] + plant species	16	167.25	0	0.67
Treatment[b] + plant species	17	169.07	1.82	0.27

[a]Slug-molluscicide and control (no slug-no molluscicide) treated as one level
[b] Slug-molluscicide and control treated as separate levels

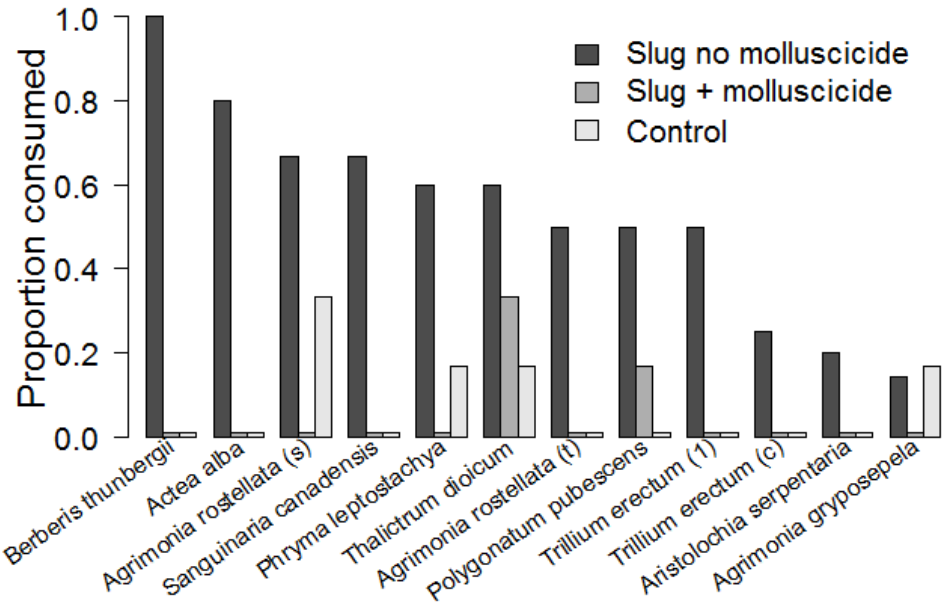

Fig. 56. Plant consumption (proportion) according to slug presence and molluscicide exposure. Species with no consumption under any treatment (*A. petiolata,* target SAR *C. retroflexa* and *Carex radiata*) were not included. Plant species were ranked according to preference. *A. rostellata* (s) grown from seed; *A. rostellata* (t) grown through tissue culture; *T. erectum* (1) were 1-leaf individuals and *T. erectum* (c) were cotyledons.

3.7.2. Earthworm effects on plant germination

Germination probability across plant species ranged from 0.007 to 0.77 (**Fig. 57**). Germination of 11 of the 19 plant species tested was negatively correlated with leaf litter biomass and no species exhibited a positive effect of leaf litter depth on germination (**Table 34**). Three species had higher germination in the south than north facing mesocosoms, while germination of the remaining species was unaffected by aspect. We found a significant interaction between leaf litter biomass and aspect on germination of two species (**Table 34**, **Fig. 58**). Because leaf litter biomass declines with earthworm activity (Suarez et. al., 2006) the negative correlation between leaf litter biomass and germination may indicate a positive indirect effect of earthworms on plant germination. Earthworms also have numerous direct effects, both positive and negative, on seed germination and seedling survival. Our data combine all these factors.

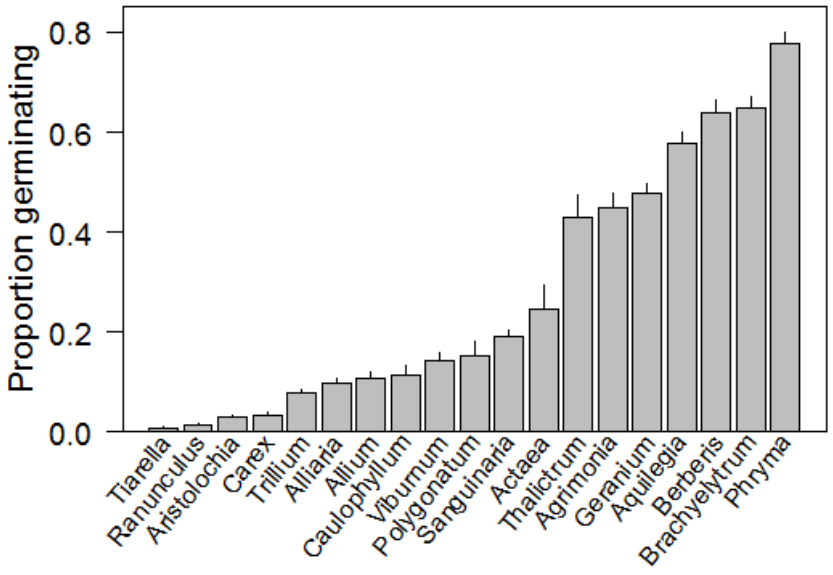

Fig. 57. Germination (proportion) of target plant species grown in mesocosms with varying earthworm density and composition.

Table 34. Model results for GLM analyses (with binomial errors) of the effects of leaf litter biomass (g) and aspect (North or South) on germination of 19 plant species. Significant terms are highlighted in bold.

Species	Leaf litter mass (g)		Aspect		Litter mass x aspect	
	t	P	t	P	T	P
Actaea alba	-2.13	**0.04**	0.97	0.34	-1.05	0.3
Agrimonia gryposepela	-2.83	**0.01**	1.83	0.07	-0.10	0.92
Alliaria petiolata	-3.86	**0**	1.02	0.31	0.39	0.70
Allium tricoccum	-5.10	**0**	-1.64	0.10	1.91	0.06
Aquilegia canadensis	1.60	0.11	1.01	0.32	-0.23	0.82
Aristolochia serpentaria	-1.45	0.15	-0.53	0.60	0.47	0.64
Berberis thunbergii	-3.29	**0**	0.58	0.56	0.06	0.95
Brachyelytrum erectum	-2.83	**0.01**	0.69	0.49	0.23	0.82
Carex radiata	-1.17	0.24	2.27	**0.03**	-0.56	0.58
Caulophyllum thalictroides	-0.78	0.44	0.55	0.59	-0.87	0.39
Geranium maculatum	-0.41	0.68	2.94	**0**	-2.36	**0.02**
Phryma leptostachya	-3.63	**0**	1.02	0.31	-0.69	0.49
Polygonatum pubescens	-2.67	**0.01**	-0.70	0.49	0.96	0.35
Ranunculus recurvatus	-1.58	0.12	-2.05	**0.04**	2.27	**0.03**
Sanguinaria canadensis	-1.45	0.15	1.10	0.27	0.03	0.98
Thalictrum dioicum	-0.36	0.72	-0.97	0.34	1.27	0.22
Tiarella cordifolia	-2.00	**0.05**	0.35	0.73	0.45	0.65
Trillium erectum	-4.96	**0**	-0.74	0.46	0.50	0.62
Viburnum lantanoides	-1.96	**0.05**	-1.12	0.27	0.74	0.46

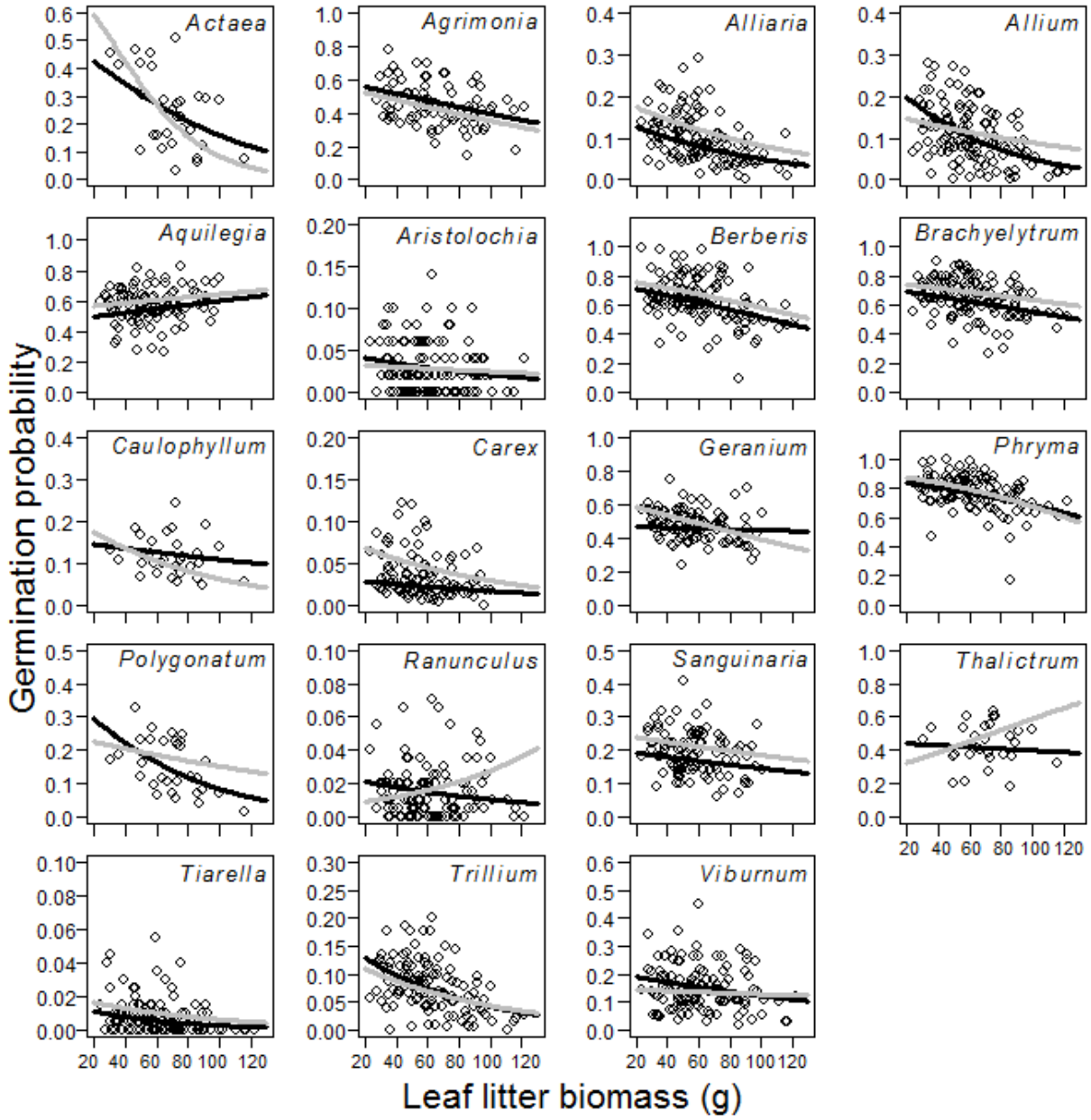

Fig. 58. Germination probability of target plant species according to leaf litter biomass (g) and aspect. Black and grey lines indicate North and South facing mesocosms, respectively. Seeds germinated in mesocosms with varying earthworm density and composition.

Survival of the six target plant species varied, with lowest survival recorded for *Viburnum lantanoides* and *Aguilegia canadensis* and highest survival recorded for *B. thunbergii* (**Fig. 59** left). Survival of four of the six species was not affected by leaf litter biomass or aspect (P>0.05 for all cases), survival of *A. canadensis* (estimate=0.018 ± 0.008, t=2.1, P=0.04) was positively correlated with leaf litter biomass and survival and *T. erectum* was negatively correlated with leaf litter biomass (estimate=-0.02 ± 0.005, t=-3.92, P=0.0001, **Fig. 59**).

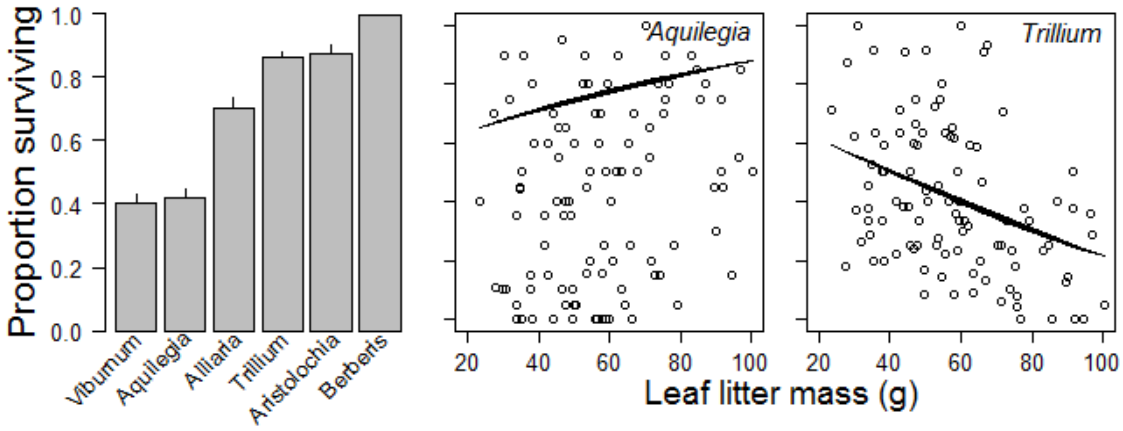

Fig. 59. Survival (proportion) of target plant species (left) and survival of *Aquilegia canadensis* (middle) and *T. erectum* (right) according to leaf litter biomass (g) at the end of the experiment. Lines depict GLM model predictions.

Leaf litter biomass had differential effect on biomass of five target species: total biomass of *Aquilegia canadensis* and *Viburnum lantanoides* were not correlated with leaf litter biomass, whereas below-ground biomass of *B. thunbergii* and above-ground biomass of *A. petiolata* and *A. serpentaria* were negatively correlated with leaf litter biomass (**Table 35**, **Fig. 60**). We found no effect of aspect (North or South) on biomass of either species.

Table 35. Model results for LM analyses of the effects of leaf litter biomass (g) on below-ground, above-ground and ratio of above- to below-ground biomass of six plant species grown in mesocosms with varying earthworm density and composition.

Species	Below-ground biomass			Above-ground biomass			Above:below Ratio		
	Estimate	SE	t	Estimate	SE	t	Estimate	SE	t
Alliaria petiolata[a]	-3.3-04	2.6-04	-1.26	-0.002	-0.0008	-2.39*	0.0002	0.0007	0.40
Aquilegia canadensis[b]	-1.75-05	1.07-04	-0.16						
Aristolochia serpentaria	-1.44-04	7.59-03	-1.91	-1.24-04	5.03-05	-2.46*	-0.005	0.007	-0.73
Berberis thunbergii	-1.31-04	4.92-05	-2.67**	-2.4-04	1.3-04	-1.78	0.001	0.002	1.05
Viburnum lantanoides[b]	1.6-04	9.1-05	0.07						

[a]For 2011 only; [b]Total biomass; *P<0.05, **P<0.01

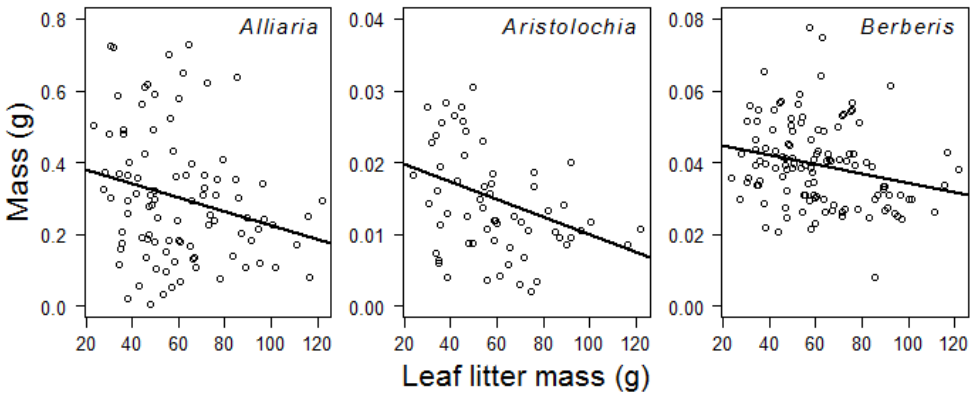

Fig. 60. Above-ground biomass of *A. petiolata* (left) and *A. serpentaria* (middle) and below-ground biomass of *B. thunbergii* (right) according to leaf litter mass (g) at the end of the experiment. Plants were grown from seed in mesocosms with varying earthworm density and composition.

3.7.3. Root-weevil and earthworm effects on plant survival

Survival of the four SAR was high (93% for *A. rostellata* and *A. serpentaria,* 98% for *C. retroflexa* and 92% for *T. erectum*) and did not vary with *B. pellucidus* or earthworm presence, expect for *T. erectum* which had lower survival in mesocosms with *L. terrestris* and *B. pellucidus* than in the remaining treatments (80% survival, estimate=-2.28 ± 1.1, z=-2.10, P=0.03).

Plant biomass was affected by treatment, but the effect depended on plant species. Above-ground biomass of *A. rostellata* and *A. serpentaria* was not affected by treatment, but *C. retroflexa* above-ground biomass was significantly lower in the *L. terrestris* mesocosms compared to the control and *Amynthas* plus *B. pellucidus* mesocosms, and *T. erectum* above-ground biomass was significantly lower in the *L. terrestris* plus *B. pellucidus* treatment than in the *Amynthas* plus *B. pellucidus* treatment (a posteriori Tukey test , **Table 36**, **Fig. 61** top). Below-ground biomass of all species was affected by treatment (**Table 36**) and was significantly lower (a posteriori Tukey test) in the *L. terrestris* or *L. terrestris* plus *B. pellucidus* treatments (**Fig. 61**, bottom). The ratio of above- to below-ground biomass was not affected by treatment (P>0.05 for all SAR).

Table 36. ANOVA results for analyses of the effects of treatment on above-ground and below-ground biomass of SAR plant species.

Species	Above-ground biomass		Below-ground biomass	
	df	F	df	F
Agrimonia rostellata	5,113	1.58	5,113	2.30*
Aristolochia serpentaria	5,113	1.89	5,113	2.64*
Carex retroflexa	5,114	3.64**	5,114	3.31**
Trillium erectum	5,107	2.37*	5,110	2.50*

*P<0.05, **P<0.01, ***P<0.001

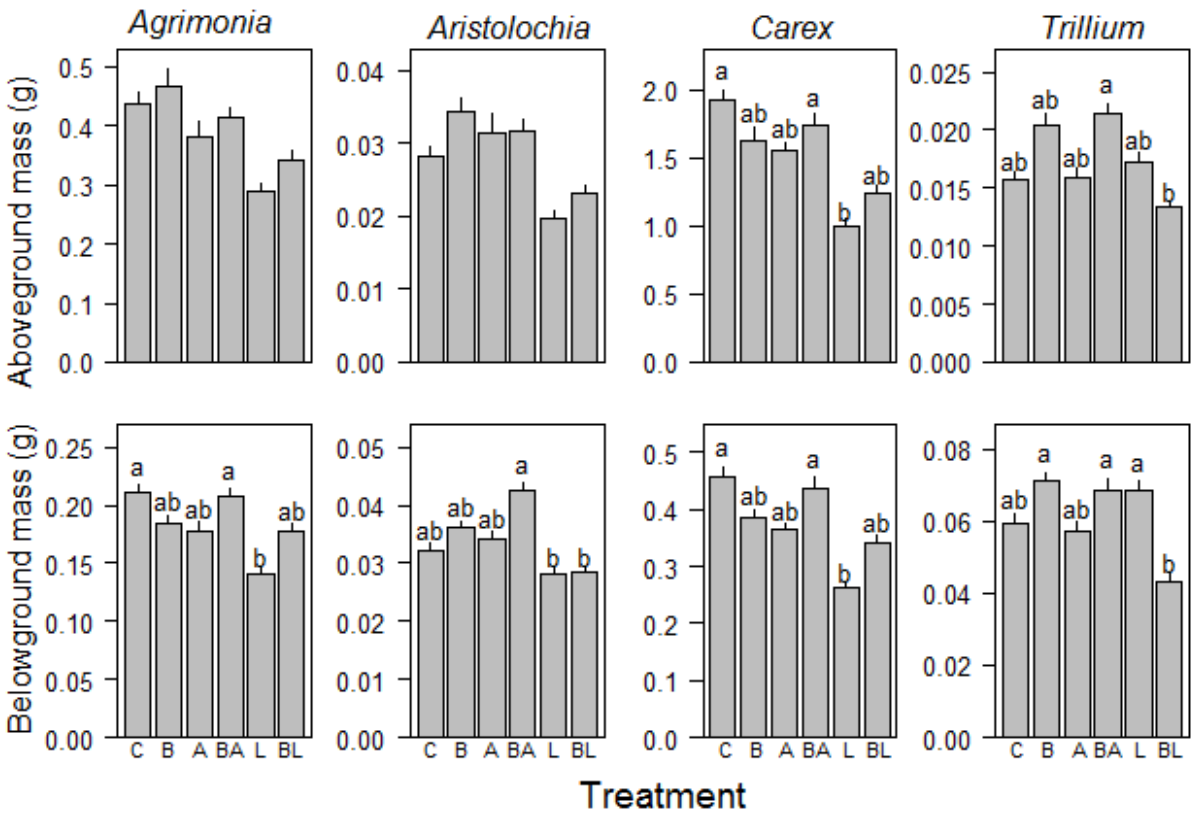

Fig. 61. Treatment effect on above- (top) and below-ground (bottom) biomass of *A. rostellata, A. serpentaria, C. retroflexa* and *T. erectum*. Treatments were control (C), *B. pellucidus* (B), *Amynthas* sp. (A), *B. pellucidus* plus *Amynthas* (BA), *L. terrestris* (L), *B. pellucidus* plus *L. terrestris* (BL). Data are means + SE, N=120. Letters indicate significant differences of the mean (a posterior Tukey tests, alpha=0.95).

Plant size was also affected by treatment, but its effect was dependent on plant species and on the variable we measured. Leaf number (6.91 ± 0.16) and leaf length (10.37 ± 0.23 cm) of *A. rostellata* seedlings were unaffected by treatment. *Aristolochia serpentaria* seedlings presented an inconsistent pattern among size variables. While leaf number (3.25 ± 0.07) was unaffected by treatment, plants were on average 1.3 cm shorter in the *L. terrestris* treatment than in the control and 2.0 cm shorter in the *B. pellucidus* plus *L. terrestris* treatment than in the only *B. pellucidus* treatment ($F_{5,112}$=6.81, P<0.001, a posteriori Tukey test). Leaves of *A. serpentaria* plants were narrower in the *L. terrestris treatment* than in the *B. pellucidus* only and *B. pellucidus plus Amynthas* treatments ($F_{5,112}$=4.10, P=0.002; **Fig. 62)**. *Carex retroflexa* had fewer culms in the *L. terrestris* only treatment than in the control, *Amynthas* and *B. pellucidus* plus *Amynthas* treatments ($F_{5,114}$=3.12, P=0.0; **Fig. 63**).

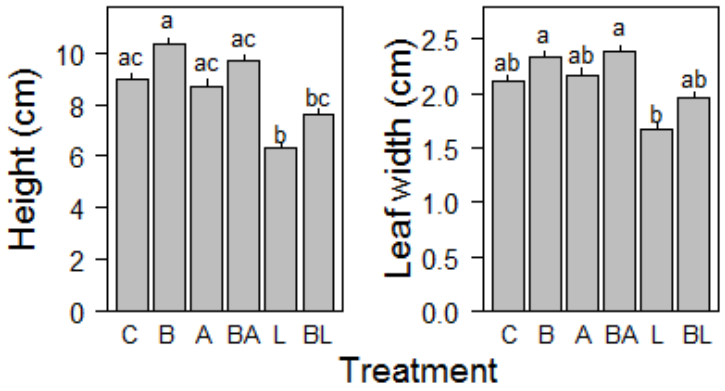

Fig. 62. Treatment effect on *A. serpentaria* height (cm) and leaf width (cm). Treatments were control (C), *B. pellucidus* (B), *Amynthas* spp. (A), *B. pellucidus* plus *Amynthas* (BA), *L. terrestris* (L), *B. pellucidus* plus *L. terrestris* (BL). Data are means + SE, N=120. Letters indicate significant differences of the mean (a posterior Tukey tests, alpha=0.95).

Only *C. retroflexa* reproduced during the study period, producing on average 10.48 ± 0.56 flowering culms per plant with 10.93 ± 0.36 seeds per culm. The proportion of flowering culms (GLMM model with binomial errors, data was not summarized by mesocosm as above) was affected by treatment and it was significantly lower in the *L. terrestris* and *L. terrestris* plus *B. pellucidus* treatments than in the remaining treatments (**Table 37, Fig. 63**). The average number of seeds per culm (LMM model with binomial errors) was also affected by treatment with lower seed production in the *L. terrestris* plus *B. pellucidus* treatment (**Table 37**, **Fig. 63**).

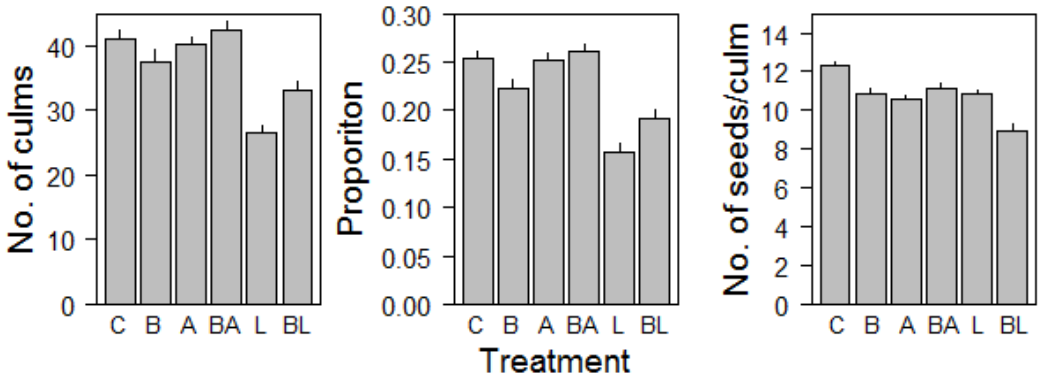

Fig. 63. Treatment effect on *C. retroflexa* number of culms (left), proportion of flowering culms to total culms (middle) and number of seeds per culm (right). Treatments were control (C), *B. pellucidus* (B), *Amynthas* spp. (A), *B. pellucidus* plus *Amynthas* spp. (BA), *L. terrestris* (L), *B. pellucidus* plus *L. terrestris* (BL). Data are means + SE, N=120.

Table 37. Model results for the effects of treatment on proportion of flowering culms (GLMM with binomial errors) and on number of seeds (LMM). Mesocosm identity was included as a random factor in all models.

Treatment	Flowering culms			Seeds per culm		
	Estimate	SE	t	Estimate	SE	t
Control	-0.92	0.08	-11	12.38	0.56	22.03
Barypeithes pellucidus	-0.08	0.12	-0.64	-1.4	0.81	-1.73
Amynthas spp.	0.07	0.12	0.6	-1.93	0.8	-2.43
B. pellucidus + *Amynthas* spp.	0.11	0.12	0.95	-1.29	0.81	-1.6
Lumbricus terrestris	-0.5	0.13	-3.95	-1.49	0.84	-1.76
B. pellucidus + *L. terrestris*	-0.27	0.12	-2.24	-2.8	0.83	-3.39

3.8. Demography model

3.8.1. *Agrimonia rostellata*

Based on *A. rostellata* natural history, we defined six stages: seed (D), juvenile (J), small vegetative (S), large vegetative (L), flowering (F) and small browsed vegetative (W) (**Fig. 64**). Small and large vegetative individuals were divided according to plant height (cutoff= 10.78 cm). We included a browsed small-vegetative stage (W) because a considerable proportion of plants became smaller after browsing and we were interested in testing whether these individuals were less likely to transition into a large-vegetative or flowering stage the next year.

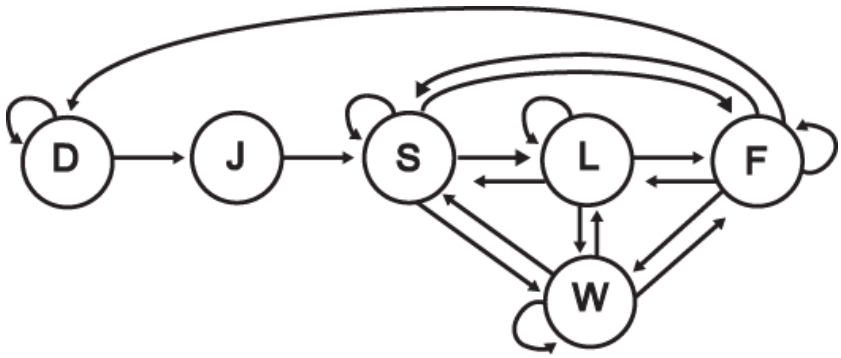

Fig. 64. Life cycle of *A. rostellata*. Letters represent different stage classes: seed (D), juvenile (J), small vegetative (S), large vegetative (L), flowering (F) and browsed small vegetative (W). Arrows represent transitions from one stage class to the next in a one-year time step.

For this species, the most parsimonious models contained main effects for transition parameters, and fencing, with an interaction between the two, but no effect of time. Consistent with our expectations, growth transition probabilities tended to be higher in the fenced than open plot, while regression transition probabilities tended to be higher in the open plot (**Fig. 65**); except for the probability of transitioning from a large to small vegetative which was higher in the fenced than open plot. As expected, the probability of remaining as a flowering individual (stasis) was higher in the fenced than open plot.

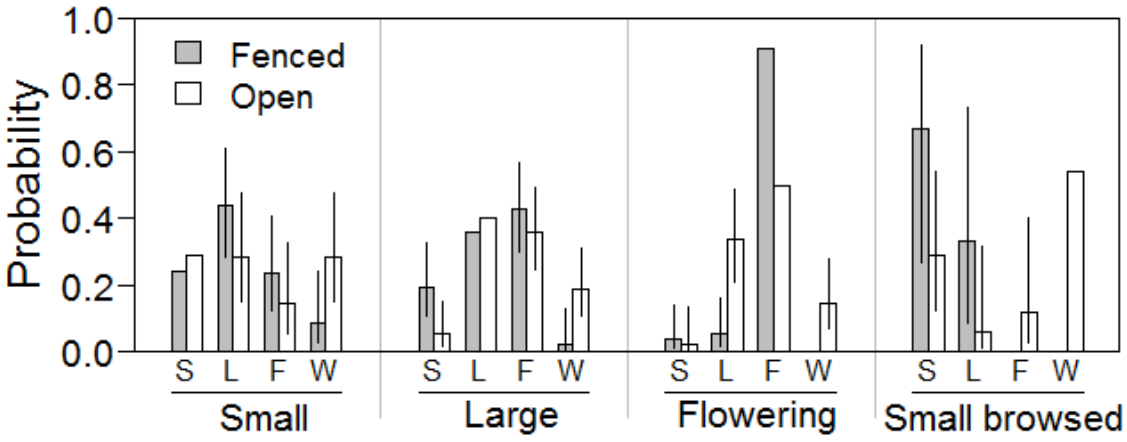

Fig. 65. Transition probabilities from (lower X axis label) and to (upper X axis label) stages: Small (S), large (L), flowering (F) and small browsed (W) of *A. rostellata* in one open and fenced plot at Blue Lake, NY. Data are means with 95% confidence intervals.

Agrimonia rostellata projected growth rate (λ) for all treatments and populations ranged from 1.319 to 1.689 indicating that populations are expected to increase (λ>1) (**Table 38**). Growth rate was higher in the fenced than open plots and slightly higher under high than low earthworm conditions.

Table 38. Deterministic population growth rate (λ) for fenced and open plots and high and low earthworm density sites.

Species	Fenced		Open		Average
	High	Low	High	Low	
Agrimonia rostellata	1.6986	1.6413	1.3430	1.3190	1.4937
Aristolochia serpentaria	1.0886	1.0881	0.8554	0.8554	0.9719
Carex retroflexa	1.8826	1.8130	1.5282	1.5541	1.6945
Trillium erectum	1.4676	1.5213	1.2939	1.3948	1.4194

Elasticity results indicated similar life cycle pathways responsible for population growth in the open and fenced plots: In both plots the most important transitions were (1) flower-flower, (2) flower-seed (fertility), (3) seed to juvenile (germination) and (4) juvenile to small-vegetative, accounting for 64% and 49% of λ, respectively (**Fig. 66**). In the open plot the transitions to- and from browsed small-vegetative (W) accounted for 17% of λ, but were almost neglible in the fenced plot.

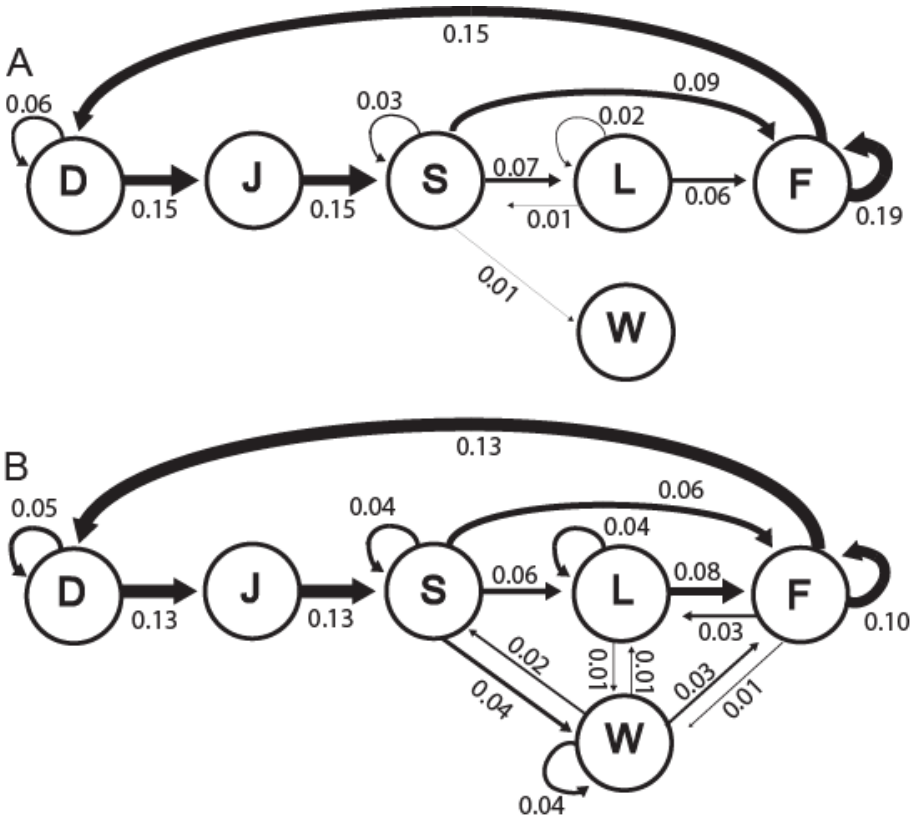

Fig. 66. Elasticities of *A. rostellata* population growth rate (λ) to changes in the entries of the annual matrices in (A) fenced and (B) open plots. Letters represent different stage classes: seed (D), juvenile (J), small vegetative (S), large vegetative (L), flowering (F) and browsed small vegetative (W). Thickness of the arrow indicates magnitude of the pathway. Only elasticities >0.01 are shown. Elasticities for populations with low and high earthworm density (not shown) presented a similar pattern.

The two-way LTRE analysis showed that the fencing effect on λ was stronger ($\lambda^m = 0.32$) than the earthworm effect ($\lambda^n = 0.024$) (**Fig. 67**). In open plots λ is reduced mainly by a decrease in seed production and by a decrease in the probability of flowering plants to remain as flowering plants. Low earthworm density reduces λ by decreasing the probability of juveniles to grow into small vegetative individuals. Thus, high earthworm density, contrary to our predictions, might have a positive, although small, effect on juvenile survival of this species, and therefore on λ.

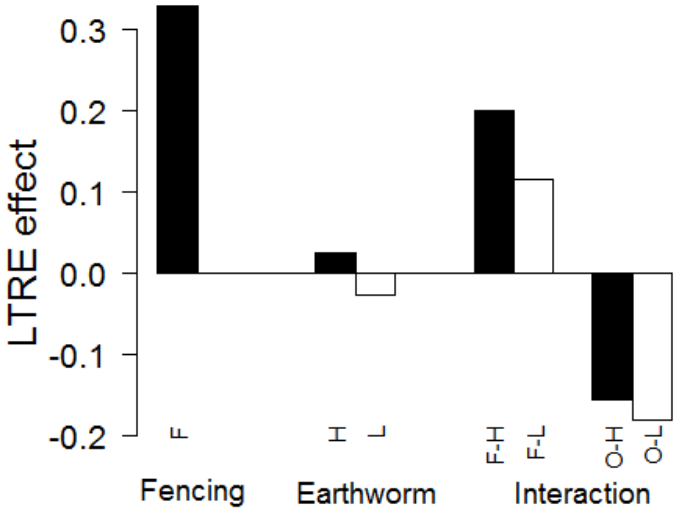

Fig. 67. Life-table response experiment (LTRE) analysis of fencing (F), earthworm density (H high density black, L low density white) and their interaction on the variation in population growth rate (λ) of *A. rostellata*

3.8.2. *Aristolochia serpentaria*

Based on *A. serpentaria* natural history, we defined five stages: seed (D), juvenile (J), small vegetative (S), large vegetative (L) and flowering (F) (**Fig. 68**). Small and large vegetative individuals were divided according to leaf length (cutoff= 4.3 cm).

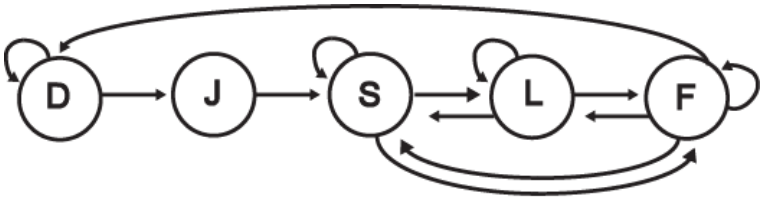

Fig. 68. Life cycle of *A. serpentaria*. Letters represent different stage classes: seed (D), juvenile (J), small vegetative (S), large vegetative (L) and flowering (F). Arrows represent transitions from one stage class to the next in a one-year time step.

For this species, the most parsimonious models contained main effects for transition parameters, and fencing, with an interaction between the two and a significant effect of time. As discussed in section 3.6.2, very few plants emerged in 2012 and only two plants flowered in the fenced plot; therefore transition estimates for transitions from this stage (flowering to small-vegetative, to large-vegetative or to flowering) are meaningless for that year. Given the data constrains, we estimated transition probabilities for 2009-2010 and 2010-2011 and results are, therefore, based on those years only.

Consistent with our expectations, regression transition probabilities tended to be higher in the open than fenced plot, with transitions from flowering to small-vegetative only occurring in the open plot. Large vegetative and flowering individuals had a higher probability of remaining in their stage (stasis) in the fenced than open plots (**Fig. 69**). Estimates for both years (2009-10 and 2010-11) were similar.

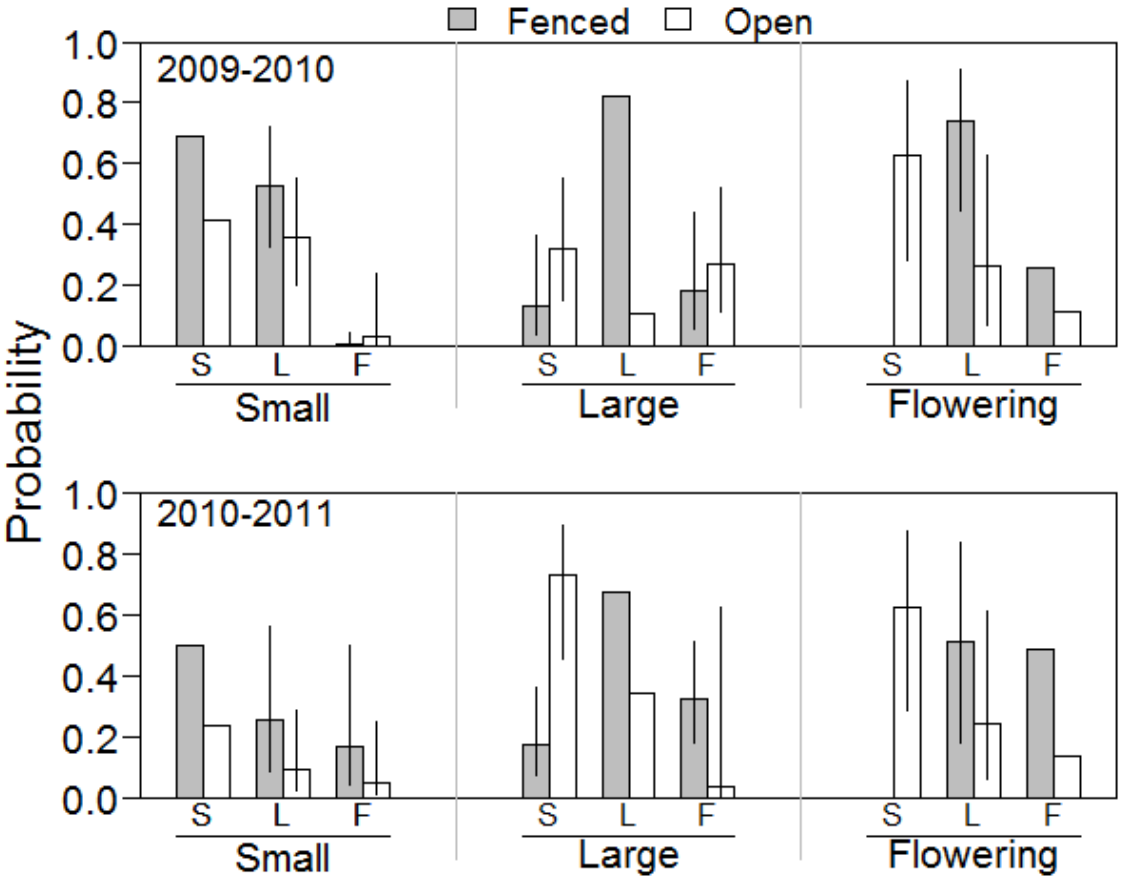

Fig. 69. Transition probabilities from (lower X axis label) and to (upper X axis label) stages: Small (S), large (L), flowering (F) and small browsed (W) in 2009-2010 (top) and 2010-2011 (bottom) of *A. serpentaria* in one open and fenced plot at West Point, NY. Data are means with 95% confidence intervals.

The projected growth rate (λ) for all treatments ranged from 0.8554 to 1.0886. In the fenced plot λ was slightly over one indicating that the population may persist; whereas in the open plot λ was below one, indicating that the population is expected to decrease (**Table 38**). Population growth rates did not differ between low and high earthworm treatments.

Elasticity results indicated a shift in the life cycle pathways responsible for population growth in the open and fenced plots: In the open plot over 99% of λ is accounted for by one pathway: seeds remaining as seeds, indicating that the presence of a seed bank might be really important for this species under high deer herbivory (**Fig. 70**). In the fenced plot, the most

important transitition was that of large-vegetative plants remaining as large-vegetative (33%), while transitions from large-vegetative to flowering and viceversa, as well as the transition from flowering to flowering accounted for another 43% of λ (**Fig. 70**).

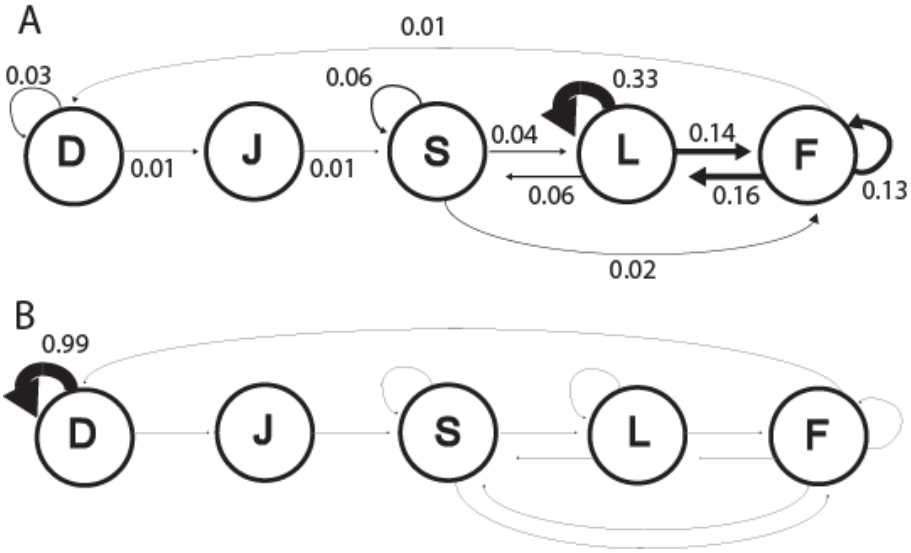

Fig. 70. Elasticities of *A. serpentaria* population growth rate (λ) to changes in the entries of the annual matrices in (A) fenced and (B) open plots. Letters represent different stage classes: seed (D), juvenile (J), small vegetative (S), large vegetative (L) and flowering (F). Thickness of the arrow indicates magnitude of the pathway. Only elasticities >0.01 are shown.

The two-way LTRE analysis showed that the fencing effect on λ was much stronger (λ^m = 0.39) than the earthworm effect, which was almost neglible (λ^n = 2.6E-05) (**Fig. 71**). In the fenced plot λ increased mainly by an increase in the probability of large vegetative to remain as large vegetative (12%) or to transition to a flowering individual (10%).

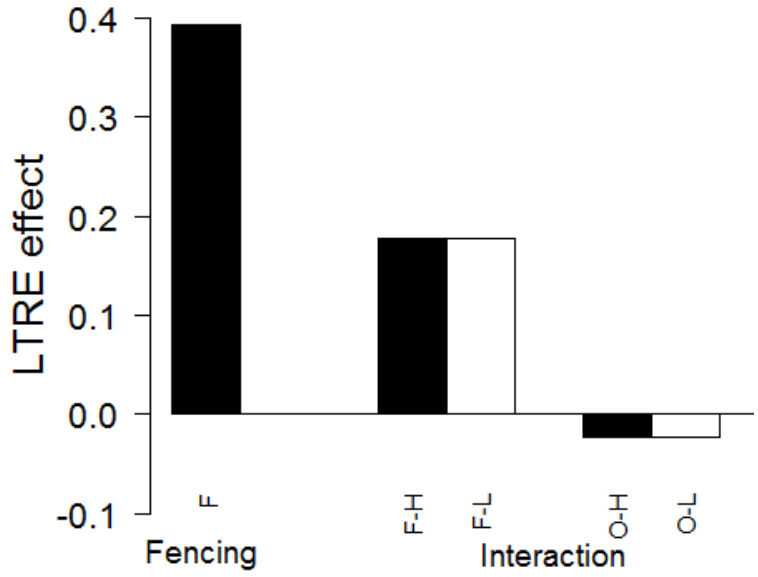

Fig. 71. Life-table response experiment (LTRE) analysis of fencing (F) and the interaction between fencing and earthworm density (H high density– black and L low white) on the variation in population growth rate (λ) of *A. serpentaria*. Earthworm effects were 2.63E-05 (not shown).

3.8.3. *Carex retroflexa*

Carex retroflexa is an evergreen sedge and therefore, we used a seasonal matrix model to account for intra-annual variation. We started by building a seasonal life cycle diagram with two phases: summer and fall. At each phase of the cycle, we chose a set of stages into which to classify individuals. Each row in the life cycle represents one phase and arrows between rows indicate how stages at one phase contribute to the next (**Fig. 72**).

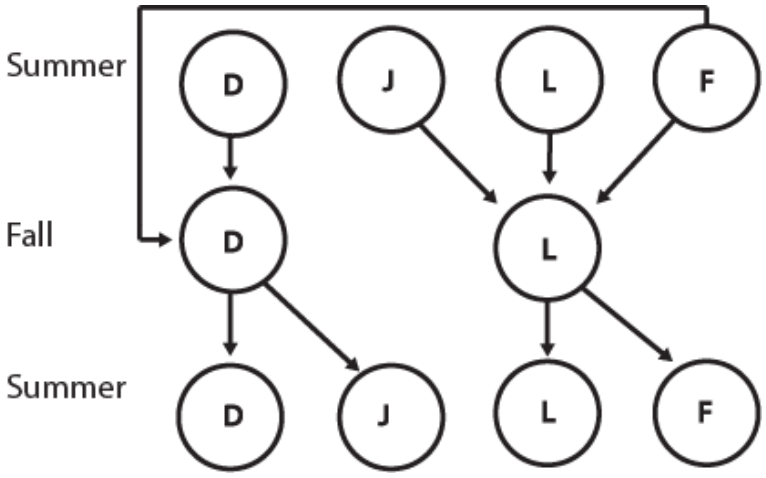

Fig. 72. Seasonal life cycle of *C. retroflexa*. Each horizontal row represents a season of the year. Letters indicate different stage classes: seed (D), juvenile (J), vegetative (L) and flowering (F). Arrows represent transitions from one stage class to the next.

Carex retroflexa projected growth rate (λ) for all treatments and populations ranged from 1.122 to 1.148 indicating that populations are expected to increase (λ>1) (**Table 38**). Population growth rate was slightly higher in the fenced than open plots. The earthworm effects depended on fencing: At fenced plots λ was slightly higher under high earthworm conditions, whereas in the open plot the opposite occurred.

Elasticity results indicated similar life cycle pathways responsible for population growth in the open and fenced plots and under low and high earthworm density (**Fig. 73**). In the summer and fall, the probability of seed to remain as seed accounted for 53% and 62% of λ in the fenced and open plot, respectively. In both plots, the transition from flowering to vegetative in the fall and from vegetative to flowering in the spring accounted for an important proportion of λ; however, those probabilities accounted for a larger proportion of λ in the fenced (30% and 38%, respectively) than open plot (19% and 28%, respectively). Although the transtiion from vegetative to vegetative (in both seasons) accounted for a small proportion of λ, its contribution was higher in the open plot.

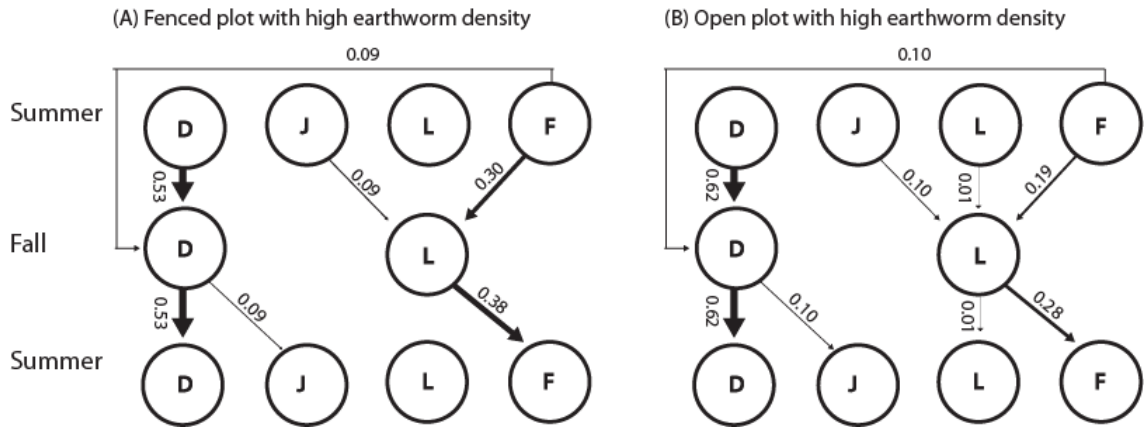

Fig. 73. Elasticities of *C. retroflexa* population growth rate (λ) to changes in the entries of the seasonal matrices in (A) fenced and (B) open plots with high earthworm density. Letters represent different stage classes: seed (D), juvenile (J), small vegetative, vegetative (L) and flowering (F). Thickness of the arrow indicates magnitude of the pathway. Only elasticities >0.01 are shown. Elasticities for low earthworm density (not shown) present a similar pattern.

The two-way LTRE analysis showed that the fencing effect on λ was much stronger (λ^m = 0.027) than the earthworm effect (λ^n = 0.002). The increase of λ in the fenced plot was due to fall to summer transitions only, specially by an increase in germination probability (7%). High earthworm density had a minor but positive effect on λ due to its positive effect on juvenile survival (0.2%), a summer to fall transition (**Fig. 74**).

It is important to note that the germination estimates were based on field trials in which we recorded very low *C. retroflexa* germination (section 3.4). Therefore, we fitted a second model using average germination under optimal conditions (average estimate=0.77, section 3.4). In the second model, growth rate ranged from 2.184 to 2.657, a higher estimate than for the previous model; but consistent with the previous model, λ was higher in the fenced than open plots and the effect of earthworm density depended on fencing. Elasticities results also indicated similar life cycle pathways responsible for population growth according to fencing and

earthworm density. However, in this model seed production and juvenile survival in the summer accounted for the highest proportion of λ (39% each), while in the previous model the probability of seeds to remain as seeds accounted for the majority of λ (**Fig. 73**). The transition from vegetative in the fall to flowering in the spring accounted for the majority of λ (60%) in this case, whereas in the previous model the transition from seed to seed accounted for the majority of λ. LTRE analysis indicated a similar effect of earthworm density for both models, but the effects of fencing were higher in the present model (λ^m=0.15) and were mostly the result of an increase in the transition from vegetative in the fall to flowering in the spring (16%).

The differences between the two models (field germination vs. optimal germination) indicates that *C. retroflexa* germination can have profound effects on demography of this species. The disparaty in germination rates in the field vs. the laboratory suggests a limitation to germination in the field, for example caused by micro-site availability, predation or pathogen effects. Given the importance of germination for this species demography, it is important to identify the reasons for low germination in the field, as well as to corroborate the recorded germination rate using a higher number of population sources and maternal lines.

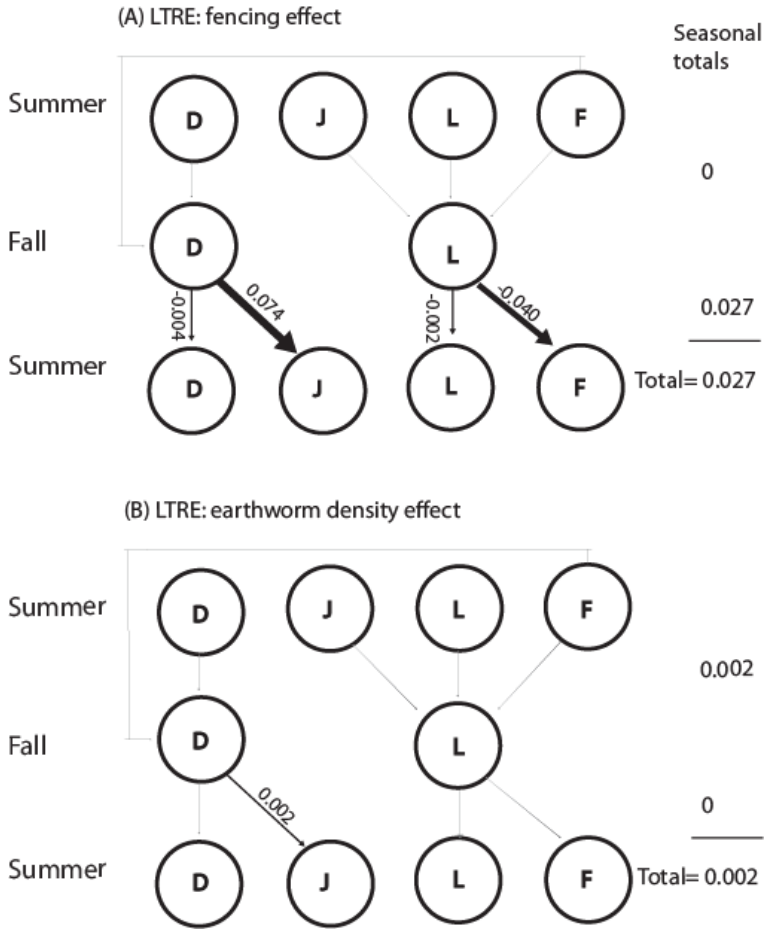

Fig. 74. Life-table response experiment (LTRE) analysis of fencing (A) and earthworm (B) effects on the variation in population growth rate (λ) of *C. retroflexa*. Letters represent different stage classes: seed (D), juvenile (J), small vegetative, vegetative (L) and flowering (F). Thickness of the arrow indicates magnitude of the pathway. Seasonal and overall total effects are shown to the right.

3.8.4. *Trillium erectum*

Trillium erectum is a long-lived perennial herb with distinct younger age classes (cotyledon and one-leaf). Based on *T. erectum* life history we constructed 7 x 7 annual stage-specific matrices for each treatment combination (**Fig. 75**). We divided small and large 3-leaf vegetative individuals according to leaf width (cutoff <5.4 cm). When building the models, we fixed certain transitions to a given value based either on the available data or on *T. erectum* biology. We assumed that 1-leaf individuals cannot transition to 3-leaf large individuals and that 3-leaf large individuals cannot regress to a one-leaf individual. We did not have enough data to pull apart annual differences and therefore results reflect a time-invariant model.

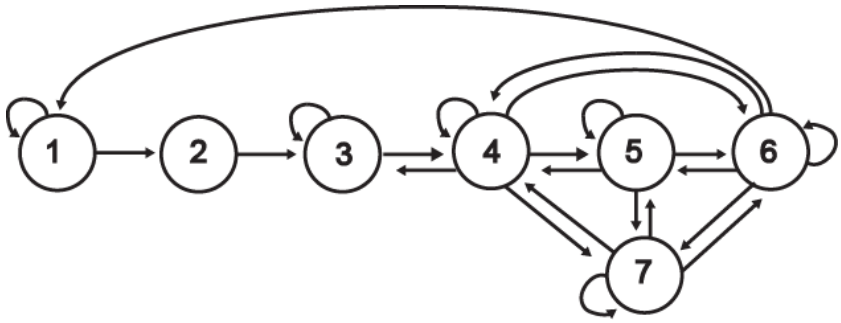

Fig. 75. Life cycle of *T. erectum*. Numbers represent different stage classes: seed (1), cotyledon (2), 1-leaf (3), small 3-leaf (4), large 3-leaf (5), flowering (6) and browsed small 3-leaf (7). Arrows indicate transitions from one stage class to the next in a one-year time step.

We found a small but apparently significant difference in survival as a function of fencing. Survival of large 3-leaf and flowering individuals was slightly higher in the open (0.969 ± 0.034 and 0.915 ± 0.061 for large 3-leaf and flowering, respectively) than in the fenced plot (0.980 ± 0.012 and 0.932 ± 0.0182 for large 3-leaf and flowering, respectively). We fixed survival of 1-leaf and of small-browsed 3-leaf to 1. It is important to note that this is apparent survival because dormancy and mortality will be confounded if a plant goes into a lengthy dormancy, for example over course of the study. At present, we are not able to account for dormancy or death in this model but hope to be able to develop data and approaches in the future ot account for these important transitions.

The best model estimated a constant detection probability for open and fenced plots and for all stages (0.936 ± 0.012). If a plant is alive, there is a (1-0.936) probability of not observing it, which would be equivalent to the probability of it being dormant, if true probability of detection for any non-dormant plant is 1. In other words, assuming that the probability of detecting a living, non-dormant plant is perfect then the probability of being dormant, irrespective of plant stage is (1-p=0.064).

Transition probabilities indicated that the probability of going from flowering to a smaller stage was higher in the open than fenced plot and, consistently, that the probability of large 3-leaf individuals transitioning into a flowering stage was higher in the fenced than open plot (**Fig. 76**). We did not detect a difference between transitions from small three-leaf individuals to other stages in response to fencing.

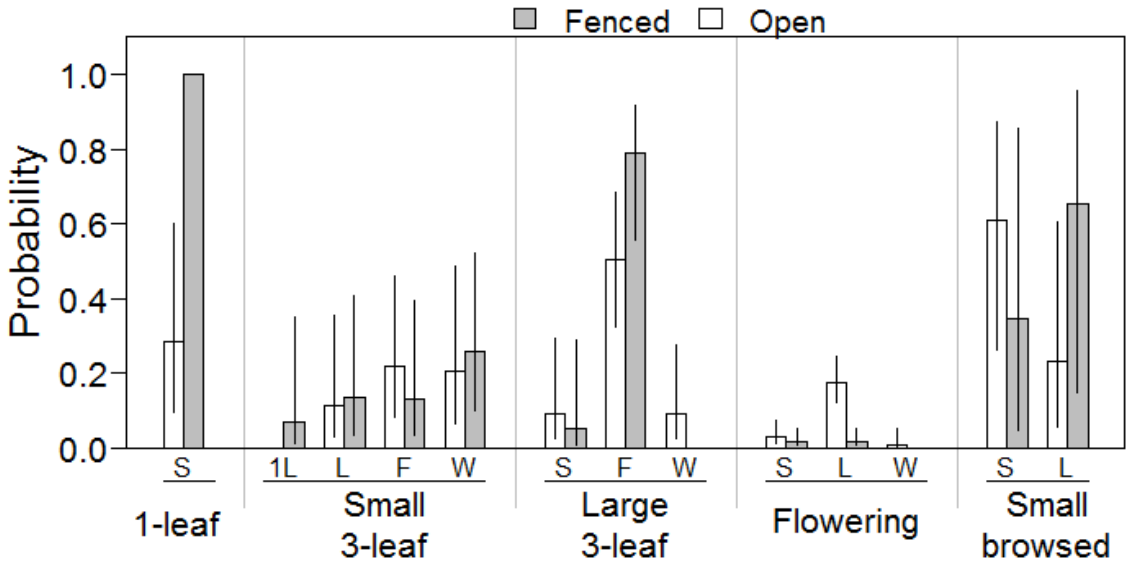

Fig. 76. Transition probabilities from (lower X axis label) and to (upper X axis label) stages: 1-leaf (1L), 3-leaf small (S), 3-leaf large (L), flowering (F) and small browsed (W) of *T. erectum* in one open and fenced plot at Richford, NY. Data are means with 95% confidence intervals.

Trillium erectum projected growth rate (λ) for all treatments and populations ranged from 1.3948 to 1.4676 indicating that populations are expected to increase (λ>1) at all plots and under low and high earthworm conditions (**Table 38**). Growth rate was higher in the fenced than open plots and higher in low than high earthworm conditions. Elasticity results indicated similar life cycle pathways responsible for population growth in the open and fenced plots, as well as in the low and high earthworm density conditions. Transitions with highest influence on growth rate were the probability of remaining as a flowering individual and of seed remaining in the seed bank (accounting aproximately for 30% of λ (**Fig. 77**)).

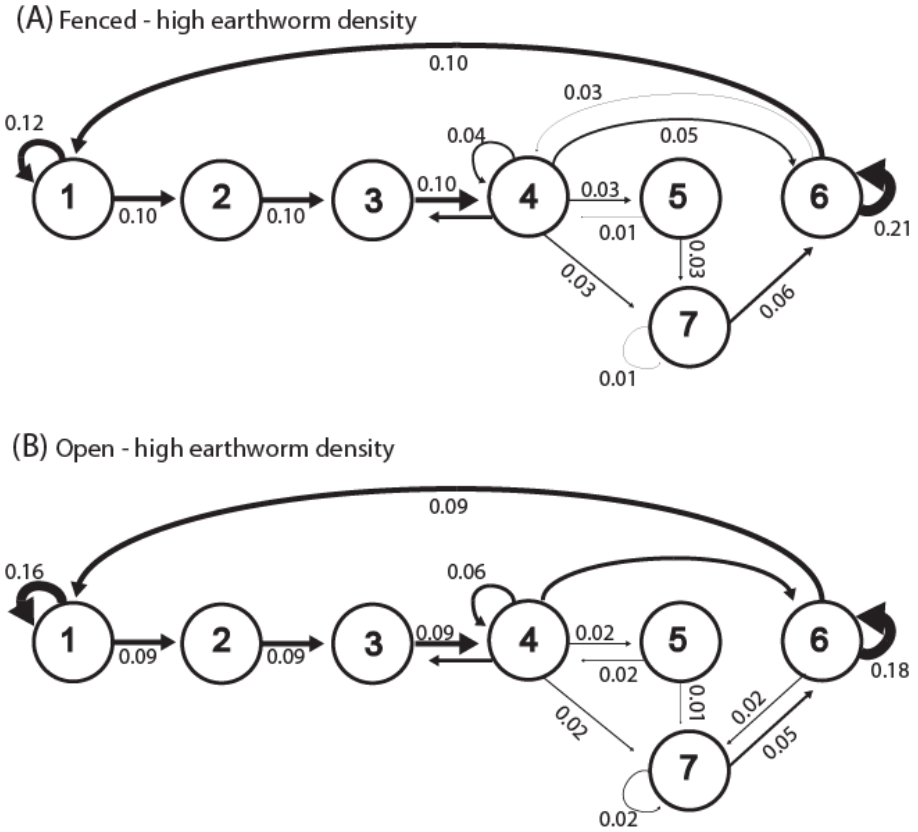

Fig. 77. Elasticities of *T. erectum* population growth rate (λ) to changes in the entries of the annual matrices in fenced (A) and open (B) plots with high earthworm density. Elasticities of plots with low earthworms (not shown) followed a similar pattern. Numbers represent different stage classes: seed (1), cotyledon (2), 1-leaf (3), small 3-leaf (4), large 3-leaf (5), flowering (6) and browsed small 3-leaf (7). Thickness of the arrow indicates magnitude of the pathway. Only elasticities >0.01 are shown. `

The two-way LTRE analysis showed that the fencing effect on λ was stronger (λ^m = 0.14) than the earthworm effect (λ^n = 0.04). In the fenced plot λ increased mainly by a increase in the probability of cotyledons to transition to 1-leaf individuals (6%) and by an increase in the probability of flowering individuals to remain in that stage (5%). Under conditions of low earthworm density, λ increased mainly due to an increase of cotyledon and 1-leaf individual survival and to their transition to the next stage. The interaction effect indicated that the effect of low and high earthworms is more prevalent in the open (λ^{mn}=0.10) than fenced (λ^{mn} =0.05) plots (**Fig. 78**).

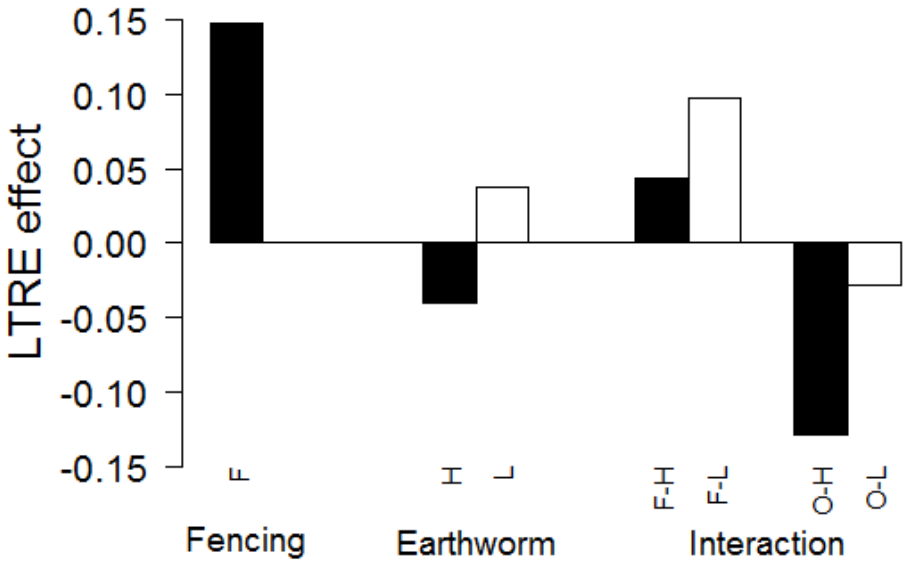

Fig. 78. Life-table response experiment (LTRE) analysis of fencing (F) and earthworm density (H high, L low) and the interaction between fencing and earthworm density (H high-black, L low-white) on the variation in population growth rate (λ) of *T. erectum*.

4. Conclusions

Our investigation of the effect of multiple stressors on demography of four different plant species has resulted in a number of important new insights. Most important, and not unexpected, is the recognition that not all plant species responded in similar ways to the different stressors. In fact our selection of different plant species aimed to cover some of the anticipated differences in responses, for example in palatability to deer, or response to earthworm invasions. However, we also discovered that different life history stages (seed germination, seedlings, juvenile or adult plants) responded in various and sometimes contradictory ways to the same stressor. For example, while earthworm invasions may facilitate germination of many plant species, established plants may be negatively affected. The important assessment then is to summarize the positive, neutral or negative effects of different stressor on overall plant demography. Despite these complications, a number of important insights can be generalized.

1. We expected that deer herbivory overrides the importance of other stressors in forest ecosystems. While deer herbivory does not appear to affect seed germination and early seedling establishment, browse sensitive species show reduced population growth rates in the presence of current deer population levels. Thus, halting, or reversing, the observed declines of many native species will be impossible without changes in deer management. Our demographic models consistently showed that deer herbivory (here assessed through fencing of individuals) for all four species in our investigation overrode effects of earthworms on plant demography.

2. The effect of non-native plants on demography of our 4 SAR species was basically nonexistent. Where we could detect an effect, it was often on germination or seedling establishment, which was improved under *M. vimineum* and thus beneficial and not detrimental to the native species we investigated. Management activities should therefore avoid focusing on non-native plants, unless a clear link to poor performance of native species has been established. Furthermore, at least for the three non-native species we investigated, the abundance of these non-native species declined, and native species abundance increased, on the inside of our deer exclosures. Thus, a "simple", but substantial reduction in deer populations alone would benefit native vegetation and reduce the presence and abundance of non-native plant invaders in forest ecosystems. The mechanisms for these effects are not entirely clear, and we assume that they are a result of a combination of direct (i.e. consumption) and indirect effects, such as through changes in competitive abilities, nutrient dynamics, microbial communities and other so-called non-consumptive effects such as trampling.

3. Earthworms change the competitive hierarchies within plant communities by changing growth and germination rates of the existing species. While our investigations were unable to reveal the long-term effects of earthworm invasions, or changes in plant communities at our research sites from a few decades ago, it is clear that competitive hierarchies are affected by presence, absence or abundance of earthworms (we found a similar effect for slug herbivory, particularly for consumption of fruits). The long-term consequences are at present difficult to assess, since earthworms interact with introduced plants, slugs and deer in complex and poorly understood ways.

   Our finding of reduced earthworm populations on the inside of our deer exclosures compared to the adjacent unfenced control plots was surprising but this

effect was maintained over the course of our investigations. This change in earthworm abundance, and hence the effect on plant germination and growth is an important consideration for management of plant communities in forest ecosystems. There is a suggestion from our work at West Point and beyond that reductions in earthworm abundance are also a function of time with further decreases in abundance the longer deer are fenced out of certain areas. The consequences of this effect, not only for plant recruitment and growth, but also for ecosystem processes such as nutrient cycling, decomposition, and water infiltration rates or nutrient dynamics, may potentially be large but require additional research. Lastly, while we can conclude that the effects of earthworms, at least on our four investigated species, should not directly result in their endangerment, previous work has clearly demonstrated the devastating effects of earthworms on litter fauna, including invertebrates and their predators such as salamanders (Maerz et. al., 2009). Thus, while plants may be able to thrive in the presence of earthworms, the continued advancement of these non-native species in forests is a conservation disaster due to their effect on litter food webs. The extent of the disappearance of specialists in litter communities is entirely unknown.

4. Our approach to assess changes in plant communities through the establishment of permanent $1m^2$ quadrats failed to detect a quick recovery once fences where erected. The positive response to deer exclusion became apparent only after five years, as native species slowly increased in abundance and non-native species decreased. Several browse sensitive species occurred in our fenced exclosures at low abundance and their positive response to deer exclusion was not captured in the permanent quadrats. We therefore measured performance of three native forbs growing inside and outside of our fenced exclosures and documented the beneficial effect of fencing on growth and reproduction of these plant species. Our vegetation monitoring demonstrates that recovery may take a very long time in areas with many years of high deer populations. We cannot exclude the possibility for evolutionary changes in plant growth and phenology due to decades of high deer abundance. We have indication for such effects for at least one plant species (early maturation at smaller sizes, similar to what is reported for effect of harvesting in fisheries or for game species).

5. There is little or no hope for restoration/recovery from the seed bank, at least not for rare species. Invasive species, particularly the annual grass *M. vimineum* show high propagule pressure and thus will likely overwhelm recovery unless deer herbivory is manipulated. We consider this lack of recovery potential the single greatest threat to forest recovery even if deer populations are greatly reduced. The lack of a seed bank, and the extremely low abundance of many species, especially late-successional herbs and species with double-dormancy, does not bode well for the long-term persistence of many species currently of conservation concern. In many Northeastern forests, including all 12 forests in this study, intense deer herbivory prevents or greatly reduces flowering and fruit production of these herbs, and thus likely contributes to the rarity of the species in the seed bank. Our germination studies show that germination rates of these species, possibly with the exception of *C. retroflexa*, are more than adequate for populations to recover, if the seed is present: Seed viability does not appear to play a major role in the rarity of the species at our sites.

The good news is, that restoration appears possible as at all sites both seeds and seedling transplants survive and grow, some becoming reproductively active in just two growing seasons. Safeguarding some of the plant species from deer and stochastic events (fire, erosion, other habitat destruction) appears paramount as a conservation

measure, particularly if only few populations exist (such as for *Aristolochia*). This will likely require establishing new populations through reseeding or transplanting efforts, potentially combined with fencing to allow populations to establish and thrive (population growth rates for all four SAR were always higher on the inside of the fence). How to do this without creating inbreeding or outbreeding depression, while accommodating impacts of global climate change, will require additional investigation.

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6. Appendices

Appendix A: Using oak sentinels to assess local deer browse pressure

Identification of the contribution of deer or deer browse pressure as a factor in determining health or plant recruitment in local forest habitats is plagued by methodological difficulties. Furthermore, current deer census methods have problems of reliability, repeatability, and interaction of density dependent and density independent factors that may affect local populations size. Moreover, deer impacts are not solely a function of deer abundance but also are associated with productivity of habitats, and legacy effects (land use history, age of forest and previous feeding pressure). Winter surveys, pellet counts, or hunter harvest numbers can rarely provide good estimates of true population size or feeding pressure. A better method is the assessment of feeding pressure and many different plant species have been proposed as indicator organisms. The most widespread and accepted method is a woody browse index where investigators focus on removal of branch tips (Morellet et al 2001). The problem with many of these browse indices is that woody browse is only one portion of a deer's diet, the frequency and biomass loss is difficult to determine, i.e. branches could be browsed multiple times which would indicate a much different feeding pressure compared to a single incidence. Similarly, regrowth and removal of regrowth are difficult to capture. Moreover, the existence/identity, diversity, distribution and abundance of different browse species plus the landscape matrix of the plot location (vicinity of clearcuts, gardens, or agricultural areas that could subsidize deer populations) influence herd size and feeding activity.

The most important question is: How many deer can an area support without severe negative consequences for native vegetation or other forest inhabitants? The answer requires reliable information about the impacts of deer on local vegetation irrespective of the estimation of deer abundance. The Sentinel procedure described below replaces unreliable deer abundance estimates, or the complicated deer woody browse survey, with a method allowing individual landowners to assess whether their local deer populations are in line with their management targets, particularly conservation-based management targets, without the need to hire a botanist or wildlife professional.

The Methodology described below uses red oak (*Quercus rubra* L.), an oak species common throughout eastern and Midwestern North America. Other tree and forb species can be used as well, and using 2 or more plant species will give a more detailed assessment of local browse pressure, as highly favored species will be browsed more intensively than less favored species. Steps 1-5 describe growing seedlings. If plants are purchased, begin with Step 6.

Materials needed:

Fig.1. Battery operated hand held drill, 2 inch or larger diameter drill bit.

Fig. 2. Red oak acorns (left), conetainers with germinating oak seedlings (right)

Fig. 3. Hardware cloth, or similar fencing material (optional) to protect seedlings; GPS unit; marking flags; individual tags (optional) to number planted seedlings

Methods

1) Collect red oak acorns (*Quercus rubra*) in the fall from local genotypes. Float acorns in water to assess viability; infected acorns float and should be discarded. Acorns (or bare root seedlings) can also be purchased.

Avoid collecting the earliest acorns that drop, as most are infested by insect larvae.. Red oak acorns are preferred since they can be easily stored over winter; acorns from the white oak group (such as White Oak and Chestnut Oak) sprout immediately after dropping and require immediate planting and overwinter storage in containers. This creates additional logistical difficulties, although they are perfectly suitable to assess deer browse pressure.

2) Store acorns over winter in a refrigerator, garage, or outside.

Store viable acorns in a mesh bag (gauze, or mesh bags used to store onions or oranges). Depending on the number of acorns to be stored, either store in a moist medium (playground sand works well, if acorns are covered with a 5cm layer of sand) or in a bag or box with some

air flow. Be sure the sand is moist but not wet. Put the container in cold storage (5°C) for the winter (in a refrigerator, garage, or outside). Keep the container covered to prevent desiccation and rodent predation.

3) Plant acorns in late winter.

Remove the acorns late February or early March from cold storage, and prepare containers for planting. The best container is 3 cm (1") diameter and 24 cm (6") deep, which allows for good root growth, compact use of growing space, and easy transport and planting (Fig. 2). Other sizes can be used but are not as efficient. Fill each container with moistened commercial potting soil, to a depth of 15-18 cm (4") and firmly compress the soil. Place one acorn in each container, then cover with 3cm (1") of moist soil, and again firmly compress the soil.

4) Grow oak seedlings.

Place containers in a greenhouse, under grow lights, or on a windowsill in a warm room and water as needed until acorns germinate. When seedlings have 2-4 leaves, fertilize with a small amount of slow release fertilizer. Keep seedlings well watered and soil moist but not wet.

5) Acclimate oak seedlings to outside temperatures.

Once seedlings are firmly established, acclimatize them to outside growing conditions to ensure that seedlings become hardy enough for transplanting. Place outside in a shady location and protect from herbivory (by rodents and deer) and make sure they are protected from late frosts. Also protect containers against earthworm colonization if transplanting into earthworm-free forests; place containers on tables/platforms in shallow outdoor pools with the bottom of the containers above water level.

6) Select planting location.

Select wooded areas free of bramble thickets and unreasonably steep slopes. Number of plantings will vary with the number of locations under investigation. In general, we plant 20 oak seedlings/ are (40 if half of them will be fenced individually). With this method we assume that we can assess deer browse pressure within an area of 20-40 ha (50-100 acres) representing a major area of a female deer territory.

7) Transplant seedlings in late spring/early summer when they are 12-30 cm tall with 4-8 leaves and have well developed root systems (Fig. 4).

The majority of deer herbivory on red oak seedlings occurs before mid-summer, so it is important to plant early. Select 20 seedlings of similar height and leaf number for each planting location. Water each seedling very thoroughly (saturate soil) before transporting to the planting site, or before planting.

Fig. 4. Oak seedling ready for transplant (left), drilling of planting location (middle), and planted seedling (right).

Plant seedlings 3-10 m apart. Drill a 20cm deep hole using a cordless drill (Figs. 1, 4), remove the seedling from the conetainer with soil attached and transfer the seedling into the hole. Removing the seedling from the container often requires gentle " massaging" of the container to loosen the soil. It is important to remove the still attached acorn before planting, otherwise squirrels, mice or chipmunks will dig up plants to retrieve acorns (Fig. 5), which often results in plant death. Plant each seedling at the same depth as in the container. Cover the potting soil with a thin (1 cm) layer of soil collected from the planting hole (to prevent desiccation) and firmly compress the soil to create good contact of rooting medium and surrounding soil. Surround each seedling with local litter to a typical site-specific depth (Fig. 4), which helps "camouflage" seedlings and reduces desiccation.

When available, record each planting location using a GPS unit to help relocate the seedlings. When using flags to mark individual planting locations (Fig. 3), avoid placing flags next to the planted seedling, which may attract curiosity by deer and thus increase herbivory levels. Place flags about a meter away from each seedling. As an extra aide, create a detailed site map showing landmarks (trees, treefalls etc.) and the location of each seedling for easier relocation of plants.

If desired, plant an additional 20 seedlings and protect them individually with a 1m tall hardware mesh cage (50cm in diameter) to exclude deer browse (Fig. 3). Stake cages to the ground using sod stakes or rebar to prevent them from being tipped over by wind or hungry deer. If desired, place an individually numbered metal label (secured with a sod stake) next to each seedling (Fig. 5).

Fig. 5. Planted oak seedling with metal tag and rodent dig to retrieve acorns

8) Revisit each site after 7-10 days to assess transplant mortality.

If well-watered before transplanting and correctly planted there should be no mortality due to transplant shock. However, if transplant mortality occurs within 7-10 days of initial planting, replace the seedling. Record mortality due to other causes or herbivory (see categories below).

9) Revisit site in September/October before leaves fall.

Prior to leaf fall in autumn (usually October) revisit planting location and record browse incidence and survival (we rarely encounter browse on our seedlings after mid August in New York). The site map is often essential to relocate planting locations if seedlings have been removed, as it is rather difficult to find a single browsed stem within other vegetation. A metal detector can help locate metal tags placed next to seedlings.

10) Revisit site in spring/early summer after leaf out to record winter browse.

Revisit planting location in mid May-June after leaf out to assess winter browse, winter survival and overall health of the seedlings. This can be done at the same time that new seedlings are planted, if desired.

11) Evaluate browse intensity

To evaluate the browse data land manger collect we need to appreciate typical seedling/sapling growth to achieve forest regeneration. It may take an individual 10-20 years to grow out of reach of a deer and even longer to get into the canopy. In many instances seedlings/saplings need to spend extended periods in the understory waiting for their chance to grow should the overstory be damaged (or harvested). Considering this early life-history, more than an occasional browse event on oak sentinels (attack on >3 seedlings) in any given year would indicate deer populations in the area as too high to achieve forest regeneration. But we see instances of 10, 15 or even more seedlings browsed in suburban areas. But some always appear to escape deer herbivory, at least initially

Furthermore, we typically see high attack on more preferred herbaceous species in places where oak sentinels are rarely, if ever, attacked while in this small size class. In areas that are of particular conservation interest, oak seedlings in their first year of growth should rarely, if ever, be attractive to deer.

Assessment details

Oak seedlings can be damaged by insects, pathogens, slugs, and rodents, in addition to deer. The following picture guide shows the most common categories of attack. The most important category to identify is deer herbivory, and the second most important issue is separating this from rodent herbivory.

Deer browse

Fig 6. Typical deer browse damage on oak seedlings (top row), where either parts of leaves or entire leaves have been removed. The lower pictures show the typical ragged edges (compared to clean, angled cuts made by rodents, see Figure 7) on woody stems and an American ginseng plant, and a seedling most likely pulled out of the soil by a deer soon after planting.

Deer browse is apparent in different ways. The most typical is the removal of some or all leaves, or part of leaves from a seedling (Fig. 6). Deer usually pull at plants/leaves creating a "rough" or "fibrous" appearance, where leaves or stems are ripped off. This is very different from rodent (Fig. 7) or insect (Fig. 8) herbivory. Many plants respond to deer herbivory by regrowing leaves, and seedlings usually do not die from a single incidence of herbivory. However, the replacement leaves are usually much smaller than the original leaves (Fig. 8), and this effect is even visible in the season following a deer browse incident.

A second sign of deer herbivory is the complete removal of a seedling. This usually occurs soon after seedlings are planted and before they have developed deep root systems. Deer tug on the leaves and pull out the entire seedling. Often the seedling is left on the ground next to the planting hole (Fig. 7).

Rodent herbivory

Rodent herbivory also comes in two different forms, one typically cutting or "felling" of the seedling (Fig. 7), the other trying to get access to the acorn (Fig. 5). The angular cut (a 45° angle) is usually clean and can be rather easily distinguished from the frayed appearance of deer browse on stems. We encounter cut stems inside and outside of our small exclosures as rodents are able to climb into small exclosures.

Fig. 7. Rodent attack showing typical angled 45° cuts (left) and a seedling cut close to the base of the stem with the top portion remaining at the planting location close to the numbered tag.

Insect herbivory and diseases

One of the most common occurrences for protected and un-protected seedlings is insect and pathogen attack by viruses and fungi. Insect and pathogen attack can come in many different ways, most often as partial removal of leaves or small holes in the leaves (Fig. 8), discoloration of leaf tissue or disease spots on the leaves. Insect herbivory can be quite extensive and entire leaves can be missing, or even defoliation of seedlings, particularly in the spring following overwintering and on replacement leaves. Many different species, including weevils and caterpillars (gypsy moth or forest tent caterpillars, for example) may be among the organisms attacking oaks but they are rarely seen.

Fig. 8. Insect attack on oak seedlings showing both " window paning" and edge feeding (left two pictures) plus seedling with replacement leaves (right) and attack but pathogens. Please note the much smaller size of the replacement leaves.

Natural mortality/wilting/senescence

Although "natural" mortality is rarely encountered, plants that are under stress (most often water stress), respond by dropping their leaves, which reduces water loss and may allow the plant to survive. Typical signs are an intact stem with buds (Fig. 9) and no signs of stem removal; the dry leaves are often scattered around the stem (Fig. 9). These seedlings may survive, but stay dormant until the next spring.

Fig. 9. Individual shoot with intact buds (left) and seedling that has dropped the leaves (right) in response to water stress.

Fig. 10. Two healthy oak seedlings after two (left) and three (right) growing seasons at West Point. Plants were protected from deer herbivory within a large fenced exclosure.

Summary and conclusion

The Oak Sentinel Approach allows landowners and land managers to assess deer browse pressure at their particular location of interest. The 'acceptable' level of browse depends on management goals. If oak reproduction or trillium preservation is important, then more than an occasional deer browse event on planted Oak Sentinels indicates an unacceptable level of browse pressure. Red oaks are an 'intermediate' preferred browse species. Species within the Liliaceae such as *Trillium* spp. are highly preferred and experience much heavier browse pressure then the oak sentinels. For example, in central NYS, both red and white trilliums (*T. erectum* and T. *grandiflorum*) can be heavily browsed (>30% of plants) by deer, while red oak sentinels at the same location remain untouched.

Current population levels of white tailed deer across much of North America are so high that forest regeneration is in jeopardy and conservation of browse sensitive species is in a crisis mode. Thus, land managers or landowners implementing the sentinel approach should not be surprised to find medium to severe browse on their planted oak sentinels at most locations. In suburban areas with deer populations exceeding 100 per square mile, 80 to 90% of planted oak individuals will be eaten within the first 2 to 3 months. This level of browse indicates that more preferred species will be eliminated at those deer densities.

If land managers consistently (over several years) find that deer browse ranges between 5-10 planted oak seedlings in each season, it likely indicates that maintaining deer populations at or close to the current level experienced by oak sentinels will not result in meaningful forest regeneration. Aggressive deer reduction measures should be taken to avoid long-term consequences of this elevated deer browse. Sites with this intermediate browse level are likely to more higher quality sites that need increased protection for conservation purposes. Once oak seedlings experience even higher browse pressure, it is likely that few conservation relevant species still exist in such locations. Additional and close monitoring of browse sensitive species (or fencing) may safeguard important source populations (or even individuals).

References cited

Morellet, N., S. Champely, J.-M. Gaillard, P. Ballon, and Y. Boscardin. 2001. The browsing index: New tool uses browsing pressure to monitor deer populations. Wildlife Society Bulletin **29**:1243-1252.